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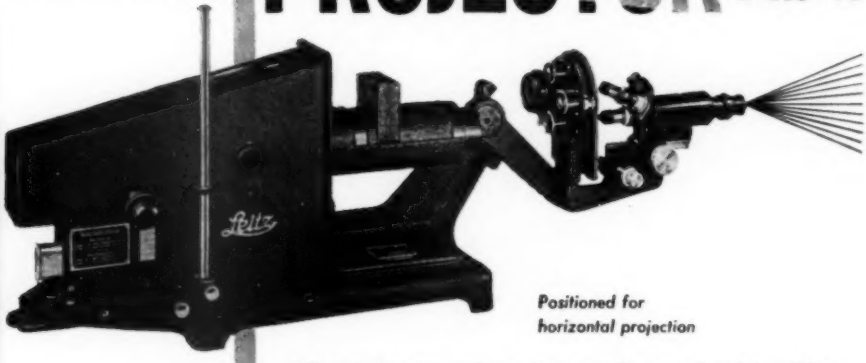


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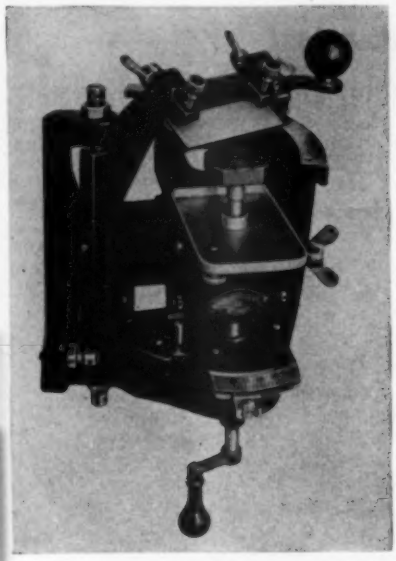

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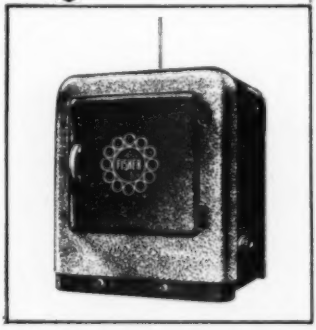

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VOLUME 52 DECEMBER 1951 NUMBER 6

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# POLYARTERITIS NODOSA

## Associated with Malignant Hypertension, Disseminated Platelet Thrombosis, "Wire Loop" Glomeruli, Pulmonary Silicotuberculosis, and Sarcoidosis-like Lymphadenopathy

W. ST. C. SYMMERS, M.D., M.R.C.P.
BIRMINGHAM, ENGLAND
AND
ROGER GILLETT, B.M., B.Ch.
OXFORD, ENGLAND

A CASE of polyarteritis nodosa which we have recently studied presented a variety of unusual pathologic features; these fell into two categories which at first sight appeared to be unrelated. The first category comprised a variety of predominantly vascular lesions. Polyarteritis nodosa with uncommonly large aneurysms, but typical in all other respects, was associated not only with the microscopic lesions of benign and malignant hypertension but also with some of the histologic appearances which ordinarily are manifestations of disseminated lupus erythematosus, while platelet thrombosis of the smallest blood vessels was also present. The second category comprised pulmonary silicosis and tuberculosis and a sarcoidosis-like lymphadenopathy. The coexistence of these various findings posed problems of correlation which have prompted us to place the case on record.

### HISTORY OF CASE

A. H. G.,[1] a man, born in 1897, had been a metal grinder for 30 years until 1947. His health was good until August, 1947, when he developed a productive cough. In November, 1947, he had slight hemoptysis; tubercle bacilli were found in the sputum. Radiography showed active tuberculosis involving the upper two-thirds of the left lung, left ventricular hypertrophy, and doubtful evidence of nodular silicosis. The family history was not noteworthy, and there was neither family nor personal history of allergic disease.

On March 1, 1948, the patient was admitted to a sanatorium. His only symptom then was a slight cough; he had had no further hemoptysis. No abnormal physical signs were found. The erythrocyte sedimentation rate (Westergren) was 5 mm. in one hour and 38 mm. in two hours. Acid- and alcohol-fast bacilli were found in sputum smears in March and April, 1948, but not subsequently, although Mycobacterium tuberculosis was isolated when the sputum was cultured in June, 1948.

---

Dr. Gillett's present address is: Department of Pathology, Radcliffe Infirmary, Oxford, England.

This case is published by courtesy of Dr. R. D. Blachford, physician, and Dr. K. S. Thompson, pathologist, Selly Oak Hospital, Birmingham, England.

Dr. Symmers is senior lecturer in pathology at the University of Birmingham Medical School and honorary consultant pathologist to the Birmingham Regional Hospital Board.

From the Department of Pathology, University of Birmingham Medical School, (Dr. Symmers) and the Department of Pathology, Selly Oak Hospital, Birmingham, England (Dr. Gillett).

1. Selly Oak Hospital No. 49/18513.

The patient was afebrile throughout his stay in the sanatorium, and he had no tachycardia. No urinary abnormality was noted. There was no clinical evidence of cardiovascular disease; the blood pressure was not recorded. He responded well to simple routine treatment. Roentgenologic examination on June 7, 1948, showed considerable resolution of the tuberculous lesions. His weight rose from 149 lb. (67.6 kg.) in March, 1948, to 153 lb. (69.4 kg.) four months later. He left the sanatorium contrary to medical advice on July 4, 1948.

In September, 1948, roentgenography showed some reactivation of the tuberculous disease in the left lung. The patient felt well and refused further treatment. Apart from slight streaking of his sputum with blood on one occasion in May, 1949, he had no symptoms of any sort until September, 1949, when he began to suffer from continuous dull epigastric pain.

On Dec. 8, 1949, he came to the outpatients' department of Selly Oak Hospital for advice about the abdominal pain. He now weighed only 127 lb. (57.6 kg.). There was no clinical evidence of pulmonary disease. The heart was enlarged to the left, so that the apex beat was in the sixth intercostal space just outside the midclavicular line. The walls of the peripheral arteries were slightly thickened. The systolic blood pressure was 220 mm. of mercury; the diastolic, 140 mm. Hypertensive retinopathy was present, with exudates, hemorrhages, and a macular fan but no papilledema. There was some epigastric tenderness, and the edge of the liver was palpable 3 cm. below the costal margin at the lateral border of the right rectus muscle; no other abnormality could be felt in the abdomen. A fractional test meal showed that the free hydrochloric acid and the total acidity of the gastric contents were within normal limits; none of the specimens contained blood, and starch had disappeared after two hours. Roentgenography after a barium sulfate meal showed marked deformity of the duodenal cap; an active duodenal ulcer without pyloric obstruction was diagnosed.

*Final Hospitalization and Further Investigation.*—The patient was admitted to Selly Oak Hospital on Jan. 19, 1950, because there had been blood in the stools (melena) for four days. He had not vomited. During the preceding few weeks his abdominal pain had become burning in character; it now occurred half an hour after eating, radiated from the hypogastrium to the epigastrium and was relieved by alkali. Apart from severe anemia, some mental irrationality, and a further reduction in weight to 104 lb. (47.2 kg.), the clinical findings were essentially the same as when he was seen as an outpatient seven weeks previously. The hemoglobin was 6.7 gm. per 100 cc.; the erythrocyte count, 1,950,000 per cubic millimeter. After transfusion of 1,700 cc. of blood the hemoglobin rose on the day after admission to 9.2 gm.

On January 25 the hemoglobin was 10.6 gm. per 100 cc.; the erythrocyte count, 3,210,000. On January 26 the blood urea was 48 mg. per 100 cc. On February 2 roentgenography showed old, active fibrocaseous tuberculosis involving the greater part of the upper lobe of the left lung and now also the subapical region of the right lung; there was also evidence of chronic bronchitis and basal emphysema; the outline of the left ventricle indicated hypertrophy of much the same degree as was seen in November, 1947; the aorta was dilated and "unwound." On February 7 the hemoglobin was 8.6 gm.; the erythrocyte count, 2,730,000; the leukocyte count, 18,000. On February 9 the blood urea was 62 mg.; the hemoglobin, 9.7 gm.; the erythrocyte count, 3,030,000; the corrected erythrocyte sedimentation rate (Wintrobe), 30 mm. in one hour. On February 10 lumbar puncture produced clear, colorless cerebrospinal fluid under normal pressure; the fluid contained 20 erythrocytes and fewer than one leukocyte per cubic millimeter; total protein, 57 mg. per 100 cc.; globulin, a trace; chlorides (as NaCl), 630 mg.; reducing substance, 55 mg.; urea, 60 mg. On February 14 the serum bilirubin was 0.7 mg. per 100 cc.; the result of a thymol turbidity test, 1.5 units (Maclagan); plasma albumin, 2.4 gm. per 100 cc.; serum globulin, 4.1 gm.; albumin-globulin ratio, 0.6; serum calcium, 8.4 mg. per 100 cc. Protein was constantly found in the urine; the specific gravity of the urine did not vary from 1.010; there was no other abnormality.

*Course.*—The patient became increasingly irrational, mentally confused and difficult to restrain. There was no history of previous mental disorder. During the last days of his illness he complained of painful sensations in the left hand, but no explanation of this was found. No abnormal physical signs were observed in the nervous system at any time. Intermittent pyrexia persisted during this final phase of his illness; the oral temperature ranged up to 101 F. (38.3 C.), and the pulse rate up to 110 per minute. The blood pressure rose to 240/160.

On February 18 the patient suddenly complained of feeling weak and became very pale. His pulse rate rose to 130 per minute, and the blood pressure fell to 90/70. He had severe loss of blood in the stools soon after collapsing. Blood transfusion was started. Further large, melenic stools were passed, and once he vomited about 100 cc. of bright blood. His condition steadily worsened, and on February 19 he died, 21 hours after collapsing.

The clinical diagnosis was acute hemorrhage from a chronic peptic ulcer of the duodenum, pulmonary tuberculosis, and arterial hypertension.

NECROPSY [2]

The body was emaciated. Only the pathologic findings are described in the following summary.

*Abdomen.*—There was an aneurysm in the pyloric region where the gastroduodenal artery crossed the duodenum. The aneurysm measured 2 by 1 by 1 cm. externally; it had a fibrous wall, 0.2 cm. thick, which was firmly adherent to the

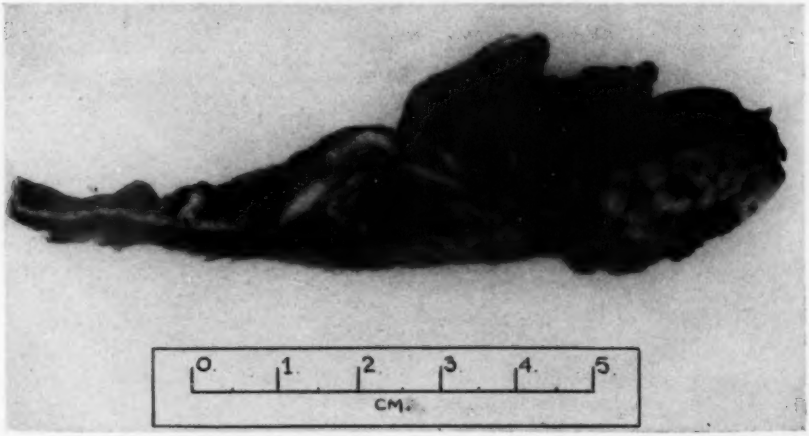


Fig. 1.—Longitudinal bisection through the gastroduodenal region to show an aneurysm (center) that has perforated into the first part of the duodenum. Part of a smaller aneurysm is shown in the head of the pancreas (right).

duodenum. Its sac contained freshly clotted blood and was lined by older thrombus. There was a shallow ulcer, 0.7 cm. in diameter, in the duodenal mucosa; the aneurysm had perforated into its floor (Fig. 1). The mucosa around the ulcer was edematous; the appearances were those of acute ulceration, and there was no sign of any active or healed chronic peptic ulcer.

The intestinal contents ranged from red blood in the duodenum and upper jejunum to typical melenic feces in the colon.

The liver was slightly enlarged. Broad, irregular patches and strands of firm fibrous tissue up to 2 cm. in width were revealed on the cut surface in the distribution of some portal tracts; here and there in these fibrotic areas there were aneurysms, 0.2 to 2.0 cm. in diameter, the walls of which were distinguished with

2. Necropsy No. S. O. H. P. M. 65/50. Necropsy was performed 48 hours after death.

difficulty from the surrounding fibrous tissue. Some aneurysms contained fresh blood clot; others contained older or organizing thrombus. The branches of the portal vein and the radicles of the hepatic veins were normal except for some compression of the former in the fibrotic areas.

Similar aneurysms arose from the pancreaticoduodenal arteries, and one was present in the head of the pancreas.

The splenic artery was tortuous and its wall greatly thickened by fibrosis.

The spleen was moderately enlarged, although its capsule was wrinkled. Bisection revealed several aneurysms (Fig. 2), ranging from 0.7 to 5.0 cm. in diameter and filled with dark red gelatinous or friable thrombus which was adherent

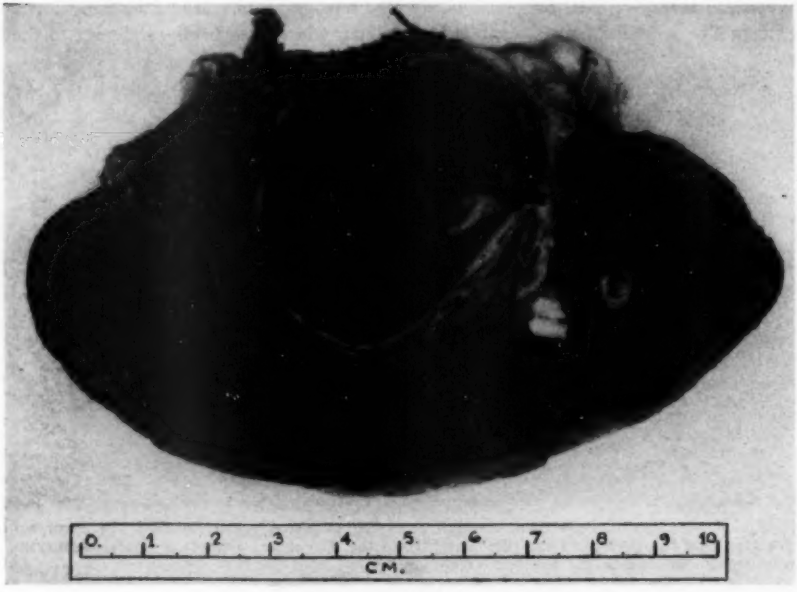


Fig. 2.—Multiple aneurysms in the spleen.

to their fibrous walls. The pulp was soft and pale, although the Malpighian bodies were prominent. There were fresh hemorrhages in the pulp, some adjacent to the aneurysms and others not apparently related to them.

The kidneys were normal in size and structural pattern, although they contained several small, irregular, pale infarcts, mainly in the cortex. The mucosa of the calices and ureters was congested.

The abdominal aorta was moderately atheromatous. There was considerable mural fibrosis of its larger branches.

The abdominal lymph nodes were unusually firm and pale but not enlarged.

*Thorax and Neck.*—There were old pleural adhesions over the apex of each lung.

The lungs were congested and moderately anthracotic and emphysematous. There was moderate peribronchial and perivascular fibrosis. A few indistinctly delimited, hard, black or gray nodules from 0.3 to 0.6 cm. in diameter were scattered through the lungs; each was surrounded by a narrow zone of emphysema. There was fibrocaseous tuberculosis of the upper half of the left upper lobe and of the apex of the right lung. The tuberculous lesions were mostly old and sharply outlined, but there were areas of caseous and gelatinous tuberculous pneumonia.

The heart was moderately enlarged through hypertrophy of the left ventricle, the wall of which reached 2 cm. in thickness. The myocardium and valves were normal.

There was slight atheroma of the thoracic aorta and its main branches.

The mediastinal and cervical lymph nodes were very firm but not enlarged.

*Cranial Contents.*—There was moderately severe atheroma of the cerebral arteries.

The only abnormality seen in the brain was a small, fresh hemorrhage between the floor of the third ventricle and the left mammillary body, partly destroying the latter.

### HISTOLOGIC FINDINGS [3]

*Pyloric Region.*—The arteries in the vicinity of the pyloric antrum and the first part of the duodenum showed simple arteriosclerosis. No acute inflammatory lesions were found in these vessels; in some, however, segments of the wall were replaced by sharply demarcated collagenous scars, and in these areas there was slight lymphocytic and plasmocytic accumulation; most of the scars had undergone aneurysmal dilatation.

Serial sections showed that the aneurysm from which the fatal hemorrhage occurred had developed as a fusiform dilatation of the proximal part of a small branch of the gastroduodenal artery. At its origin this branch had a mean external diameter of 0.2 cm. Close to its origin the normal tissues of its wall were replaced by an everted fibrous scar that merged with the fibrotic wall of the aneurysmal sac, which was devoid of muscle and elastic elements; the sac contained organizing, compact, hyalinized thrombus peripherally and fresh clot centrally. There was an early, acute ulcerative inflammation of the duodenal tissues around the breach in the wall of the sac; there was no evidence of active or healed chronic peptic ulceration. Similar but smaller aneurysms had developed from other small arteries adjacent to the bowel wall.

The arterioles showed a variety of changes. Severe hyaline hypertensive arteriolosclerosis affected many of those with an external diameter of about 40 microns or less, and there was hyperplastic sclerosis of the larger arterioles. Lesions characteristic of various stages of the development and healing of polyarteritis nodosa were seen in small arteries and arterioles, and vascular changes of the type which is more characteristic of disseminated lupus erythematosus were also present; as lesions of both types were more numerous at all stages in the liver, their histologic appearances may more conveniently be described under the heading of liver.

3. The tissues were fixed in formaldehyde-saline solution (4% formaldehyde). Paraffin sections were stained by various methods.

Some precapillary arterioles, capillaries, and smallest-caliber venules were occluded by amorphous hyaline or granular thrombi in which no cells were identifiable except for an occasional erythrocyte or leukocyte in a few of the granular ones (Fig. 3). Thrombocytes could not be demonstrated unequivocally, although Giemsa's stain and phosphotungstic acid-hematoxylin revealed in the granular thrombi a fine blue stippling which may have represented platelets. There was endothelial proliferation in some of the vessels containing these thrombi; in others the thrombus was seen to be adherent to areas whence endothelium had disappeared. The surface of some propagated thrombi had become covered with a layer of

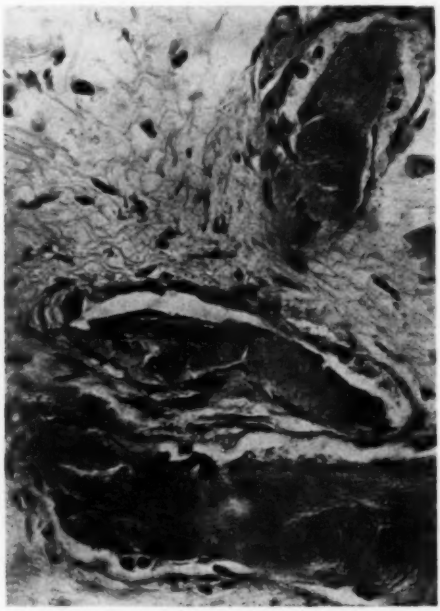

Fig. 3.—Platelet thrombi distending a group of small blood vessels in the periduodenal tissue. There is partial endothelialization of the surface of the thrombi. Hematoxylin-eosin; × 200.

endothelial cells. Some of the older thrombi were organizing. No hemoglobin, iron, or hematoidin could be demonstrated in the thrombi. A few fine fibrinous threads were demonstrable in some granular thrombi but not in the hyaline ones. Many affected arterioles were so distended and their walls so attenuated that they could be differentiated from venules only by examination of serial sections; unequivocal venules were, however, occasionally involved.

*Liver.*—Chronic passive engorgement of the liver was associated with moderate centrilobular atrophy. In some lobules the parenchymal cells contained fat vacuoles. The lobular pattern was not distorted except for compression in the vicinity of the aneurysms; in these areas there were also small foci of parenchymal necrosis, some of which were demonstrably due to polyarteritic anterior occlusion. Glisson's cap-

sule, except for the broad fibrotic zones noted macroscopically, was normal, although there were sclerosis of the muscular arteries and marked hypertensive hyalinization of the smallest arterioles.

The areas of macroscopic fibrosis consisted of collagenous tissue of varying density, sharply delimited from the surrounding parenchyma. The connective tissue fibers and the interstitium showed no unusual appearances. Fibroblasts and inflammatory cells were scanty except in the vicinity of arteritic lesions. Occasional adenomatoid congeries of bile ductules were present. The branches of the portal veins were normal except for some distortion caused by the surrounding fibrosis. The arteries were strikingly abnormal. The largest branches of the hepatic arteries showed muscular hypertrophy, multiplication of elastic laminas, and fibrosis. Aneurysms similar to those in the pyloric region were present on some of these vessels and on adjacent segments of their branches. They ranged from 0.1 cm. or less up to 1.8 cm. in diameter, and some could be seen to have formed as saccular outpouchings of fibrous scars in the vessel wall (Fig. 4); serial sections showed that others were fusiform dilatations of smaller fibrosed vessels.

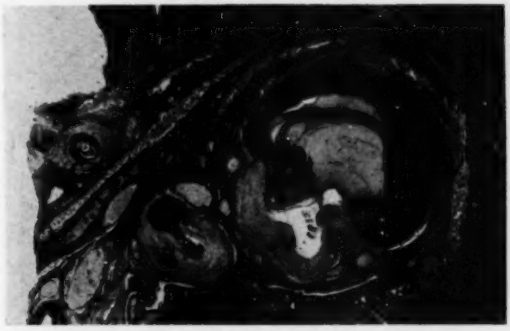

Fig. 4.—Thrombosed saccular aneurysm of an artery (*A*) in a portal tract. A smaller, organizing aneurysm is also present (*B*), and a small artery (*C*) shows acute polyarteritis with a dense ring of fibrinoid. Van Gieson's stain; × 5.5.

Changes typical of polyarteritis nodosa were present in many arterioles and small arteries; vessels thus affected were found only in the areas of macroscopic fibrosis. The earliest stage of this process was edema and scanty accumulation of neutrophil segmented granulocytes and lymphocytes in the vessel wall. In some of the earliest lesions the muscle cells were swollen and homogenized or had degenerated into refractile, intensely eosinophil fibrinoid. In more severely involved vessels there was fibrinoid necrosis of part or all of the media as well as a great accumulation of inflammatory cells, predominantly neutrophils, in all coats and in the perivascular tissue (Fig. 5). Eosinophil granulocytes were not numerous. In most instances the endothelium was intact and the lumen contained normal blood; other vessels were thrombosed. In some instances the site of the vessel was shown only by a dense accumulation of inflammatory cells in and around an irregular ring of fibrinoid. In the smallest vessels necrosis and inflammation involved the whole circumference, but in the larger ones, usually only part. In older lesions lymphocytes, plasmocytes, and histiocytes took the place of segmented granulocytes, and

later fibroblastic proliferation led to concentric fibrosis with obliteration or partial recanalization of the lumen; other affected vessels became replaced by an irregular mass of almost acellular, hyaline tissue.

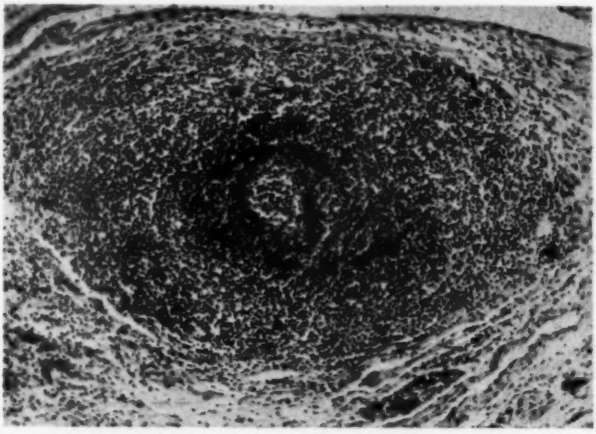

Fig. 5.—Acute polyarteritis affecting all coats of the wall of a small subserosal artery in the duodenum. Hematoxylin-eosin; × 86.

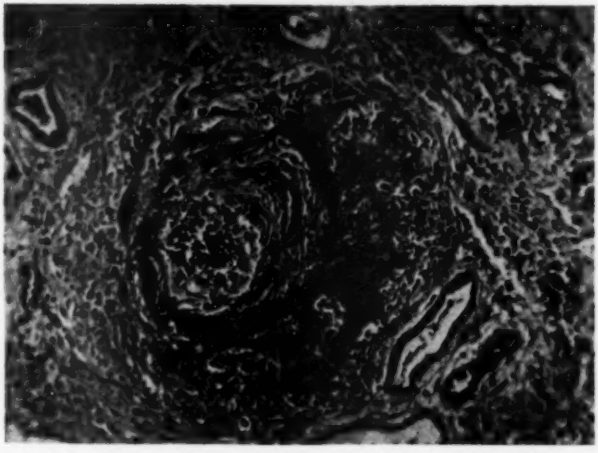

Fig. 6.—Disseminated lupus erythematosus type of arteriolar lesion in kidney. There is widespread fibrinoid alteration of connective tissue fibers of the vessel wall, with little cellular reaction. Hematoxylin-eosin; × 160.

In addition to the changes already described there was a less common arteriolar lesion, indistinguishable from the characteristic vascular lesion of disseminated lupus erythematosus. The earliest stage of this lesion was an accumulation of fibrinoid material in one or more coats of the vessel wall; this was accompanied by pro-

liferation and degeneration of fibroblasts, particularly in the intima, so that the lumen became greatly narrowed, although remaining patent. Atrophy and disappearance of the elastic and muscular elements followed progressive replacement of the fine fibrous framework of the vessel wall by increasing amounts of fibrinoid. There was remarkably little cellular reaction in vessels showing this lesion (Fig. 6).

Platelet thrombi were present in a few of the finest-caliber arterioles and capillaries in the portal tracts. None was found in the hepatic sinusoids or venules.

*Pancreas.*—There was moderate interlobular fibrosis. The parenchyma was normal. All the vascular changes seen in the liver were represented.

*Splenic Artery.*—The wall of the splenic artery was greatly thickened by fibrosis which affected predominantly the intima.

*Spleen.*—The prominence of the Malpighian bodies on the cut surface of the spleen (Fig. 2) appeared to have been due to thickening of the central arterioles,

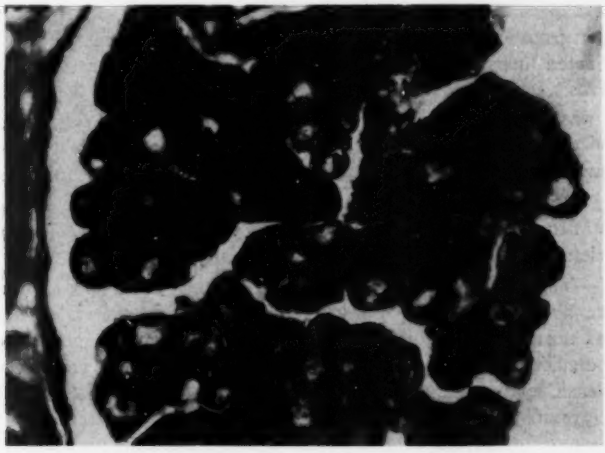

Fig. 7.—"Wire loop" lesion in a glomerulus. Hematoxylin-eosin; × 456.

around many of which there was a striking concentric fibrosis of the "onion peel" type seen in disseminated lupus erythematosus. Other vessels showed advanced mural hyalinization. Platelet thrombi were present in a few arterioles. The sinusoids were engorged, and there were small, fresh peripenicillary hemorrhages.

The collagenous walls of the false aneurysms were distinguished from the trabeculae by their disposition and structure, particularly the absence of elastic fibers. The aneurysms contained fresh or old thrombus, and there was hemorrhage around some of them.

*Kidneys.*—The general structure was preserved. In some glomeruli there were adhesions between the capillary tufts and the capsular parietes. The capillary basement membrane was irregularly thickened and hyaline in many glomeruli, the appearances being those seen in benign nephrosclerosis; the hyaline material gave the staining reactions of collagen. In a small number of glomeruli a morphologically and tinctorially different type of thickening of the basement membrane of some or

all of the capillary loops presented a picture which was identical with the characteristic "wire loop" appearance seen in disseminated lupus erythematosus (Fig. 7). The basement membrane of these "wire loop" vessels was more eosinophil than in other glomeruli; it stained red or pink with Mallory's connective tissue stain and did not give the collagen coloration with Van Gieson's stain or Masson's trichrome stain. No thrombiform masses were seen in any "wire loop" capillaries. Typical platelet thrombi were present in some other glomeruli and in intertubular capillaries, arterioles, and venules.

The afferent glomerular arterioles showed marked hyalinization and sometimes fibrinoid necrosis with sparse local accumulation of neutrophil segmented granulocytes and occasional small hemorrhages. Fibrinoid necrosis often also involved part or all of the related glomerulus, and the appearances were typical of those seen in malignant hypertension.

Typical changes of polyarteritis nodosa were also present. There were small hemorrhagic or ischemic infarcts in the cortex, many of them due to polyarteritic occlusion. A considerable number of arterioles showed the distinctive vascular lesion of disseminated lupus erythematosus (Fig. 6). The larger arteries showed simple arteriosclerosis.

There was more or less cloudy swelling and hyaline droplet degeneration of the renal tubules, some of which contained hyaline or erythrocytic casts.

No amyloid was demonstrable with Congo red or metachromatic staining methods.

*Skeletal Muscle.*—The muscle fibers were normal. There was some hyperplastic arteriolosclerosis, and an occasional platelet thrombus was found.

*Lungs.*—The pleura was fibrotic, especially over the upper lobes; some collagen fibers were hyalinized, but no fibrinoid change was present and the interstitial matrix was normal. There was extensive tuberculosis in the upper lobes. Much of the tuberculous process was of old standing; these lesions consisted of broad zones of dense, hyaline fibrous tissue enclosing areas of caseation, with little or no remaining granulomatous tissue. Elsewhere, however, this chronic reaction gave place to a typical necrotizing acute tuberculous bronchopneumonia and bronchiolitis. A few acid-fast bacilli with the typical morphology of Mycobacterium tuberculosis were found, singly or in small groups, both in the old caseous areas and in the necrotizing acute lesions.

Silicotic nodules up to 0.35 cm. in longest dimension were scattered sparsely through the lungs. They consisted of hyaline whorled fibrous tissue which in the larger nodules surrounded a central zone of granular necrotic debris in which the outlines of the preexisting tissue structure remained recognizable in places. The nodules contained much carbon and also finely scattered minute granules of refractile brown-yellow dust, which was in part birefringent and which withstood microincineration at 600 C. for one hour (silica). In many nodules occasional tubercle bacilli were demonstrable.

Arteritic lesions in the lungs were limited to acute tuberculous arteritis in the areas of tuberculous pneumonia. No platelet thrombi or megakaryocytes were seen in the lungs.

*Lymph Nodes.*—All the nodes examined (cervical, mediastinal, and numerous abdominal nodes) were histologically similar (Fig. 8). As a result of very exten-

sive hyalinization of the stroma, the lymphoid tissue had been greatly attenuated. The hyaline tissue was uniformly eosinophil and fuchsinophil and was colored deep blue by Mallory's connective tissue stain; no paramyloid or amyloid was found. Most sinuses were obliterated by sclerosis; in those which remained patent there was some littoral cell hyperplasia and histiocytosis, and an occasional small rounded aggregate of large epithelioid cells, sometimes with one or two multinucleated giant cells. Scanty multinucleated giant cells were also scattered through the nodes in relation to typical Schaumann corpuscles.[4] A few of the giant cells contained a single, typical asteroid body.[4] There was no necrosis. The findings were identical with those of the late sclerotic stage of sarcoidosis. No comparable changes were found in any other organ. No silicotic lesions were found in the lymph nodes, and no siliceous material could be demonstrated in them by microincineration and chemical studies.

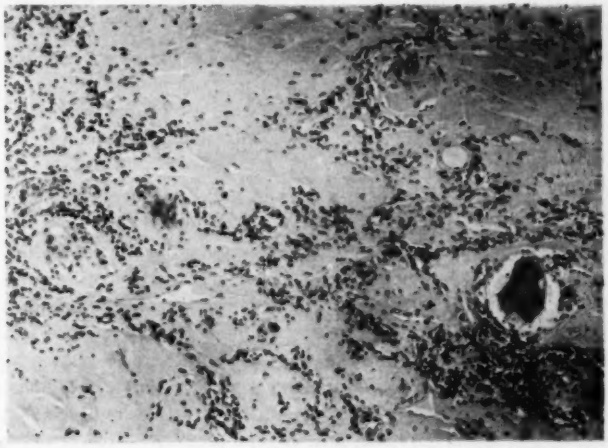

Fig. 8.—Lymph node showing extensive hyalinization with surviving giant cells. Hematoxylin-eosin; × 100.

*Brain.*—The hemorrhage which had partly destroyed the left mammillary body had originated from a false aneurysm identical with those in other organs. The hemorrhage was partly fresh and partly old. The walls of many capillaries in the neighborhood of the aneurysm were impregnated with intensely hematoxyphil material which gave a strongly positive iron reaction. Calcific (von Kossa-positive) or iron-impregnated spheruloids were also numerous in this region. The neurons, glia, and vasculature of the right mammillary body and of the surviving part of the left one were normal.

Platelet thrombosis partly or completely occluded a number of the smallest-caliber vessels in many parts of the brain. There were no other vascular lesions.

*Basilar Artery.*—Sections of the basilar artery showed only atherosclerosis.

4. Symmers, W. St. C.: Localized Tuberculoid Granulomas Associated with Carcinoma: Their Relationship to Sarcoidosis, Am. J. Path. **27**:493-521 (May-June) 1951.

## COMMENT

### Polyarteritis Nodosa

The diagnosis of polyarteritis nodosa in this case appears to be indisputable. All stages of development and healing of the characteristic lesions were recognizable. No comparable phlebitic condition was found. No giant cell arteritic lesions [5] and no "allergic granulomas" [6] occurred.

Areas of infarction large enough to be seen by the unaided eye were present only in the kidneys. There were microscopic foci of necrosis due to polyarteritic vascular occlusion in the liver.

*Aneurysms.*—Arterial aneurysms, which ranged in size from microscopic dimensions up to 5 cm. in diameter, were present in the gastroduodenal region, liver, pancreas, spleen, and brain, and had developed following polyarteritic necrosis or postnecrotic fibrosis of the vessels. Some were saccular or fusiform dilatations of medium-sized arteries. Others, such as the large one from which the fatal duodenal hemorrhage had occurred, were shown by serial sections to have arisen from a small vessel close to its origin from a large artery, and not from the latter itself as had seemed macroscopically the case. Acute polyarteritic lesions of small vessels were often found in that part of their course, just beyond their origin, which corresponded with the site of predilection for the formation of aneurysms on other vessels of like size.

With the exception of very small lesions in which remnants of the vessel wall still bounded the sac, the aneurysms were of the so-called false variety. All contained thrombus, mostly of recent date. The appearances of the aneurysms indicated that increase in their size was partly due to hemorrhage from them into the surrounding tissues with subsequent peripheral organization of the hematomas. Fresh hemorrhages were present in the spleen and the brain. While arterial hypertension must have been an important factor in the causation of these hemorrhages, it is unlikely that spontaneous cessation of bleeding would have occurred unless the point of leakage was protected from the full force of the greatly raised blood pressure by partial thrombosis of the aneurysmal sac.

There was great fibrous thickening of some portal tracts, apparently secondary to the local vascular disease. Christeller [7] suggested that such macroscopic appearances were diagnostic of polyarteritis nodosa, and he named the condition interstitial periarteritic hepatitis. The only condition known to us which is likely to be confused macroscopically with this periarteritic hepatitis is the "pipe stem" variety of schistosomal cirrhosis.[8]

The aneurysm from which the fatal hemorrhage occurred abutted on the wall of the first part of the duodenum and may well have been responsible for the dyspeptic

5. Harrison, C. V.: Giant-Cell or Temporal Arteritis: A Review, J. Clin. Path. **1**:197-211 (Aug.) 1948.

6. Churg, J., and Strauss, L.: Allergic Granulomatosis, Allergic Angiitis, and Periarteritis Nodosa, Am. J. Path. **27**:277-301 (March-April) 1951.

7. Christeller, E.: Ueber die Lokalisationen der Periarteritis nodosa, besonders in den Bauchorganen, Arch. Verdauungskr. **37**:249-273, 1926.

8. Symmers, W. St. C.: Note on a New Form of Liver Cirrhosis Due to the Presence of the Ova of Bilharzia Haematobia, J. Path. & Bact. **9**:237-239, 1904.

symptoms and the roentgenologic appearances simulating chronic peptic ulceration. No active or healed chronic peptic ulceration was found at necropsy. It is of interest that Christeller recorded a case of polyarteritis nodosa in which death was due to rupture of a thrombosed [sic] aneurysm of a pancreatic branch of the celiac axis into the duodenum.[7]

*Arterial Hypertension.*—It is well known that polyarteritis nodosa is often associated with arterial hypertension. In our case hypertension was associated with severe systemic arterial and arteriolar sclerosis and fibrinoid necrosis of afferent glomerular arterioles and glomeruli. There was no severe renal failure.

Although roentgenograms showed left ventricular hypertrophy, cardiovascular disease was not observed clinically while the patient was in the sanatorium two and a half years before his death; there is no record of the blood pressure then. As the necropsy revealed no other cause for the cardiac enlargement, this was assumed to be due to "essential" hypertension of at least two and a half years' duration, although it is likely that polyarteritis nodosa caused the great elevation of blood pressure observed during the last two and a half months of life. There appear to be no grounds for suggesting that hypertension in this case was entirely secondary to polyarteritis nodosa, of which the first clinical evidence was the onset of abdominal symptoms five months before death.

It has been asserted that hypertension is a factor in the causation of polyarteritis nodosa, and that the latter is to be distinguished clinically and pathologically from allergic necrotizing panarteritis ("hypersensitivity angiitis").[9] In respect to this we shall mention here only the observation that arterial hypertension may be absent throughout the course of an illness in which necropsy reveals a picture conforming with the pathologic criteria of polyarteritis nodosa as described by Dr. Zeek and her co-workers,[9] or may first appear at any time, even after a histologic diagnosis has been made. We do, however, admit that hypertension appears to be the major etiologic factor responsible for polyarteritis-nodosa-like lesions which may be found, limited to the pulmonary arterial tree, in cases of pulmonary hypertension [10]; nevertheless, there is much evidence against the general applicability of the hypertensive hypothesis of the causation of polyarteritis nodosa.

### Platelet Thrombosis

Another vascular abnormality in our case was the presence of disseminated lesions which were identical, except in their rather less frequent occurrence, with those of thrombotic thrombocytopenic purpura.[11] It is not claimed that this was a case of thrombotic thrombocytopenic purpura *sine* purpura. No manifestations unequivocally due to platelet thrombosis were detected during life. The mental disorder, fever, anemia, and leukocytosis, while consistent with the platelet thrombosis syndrome, were adequately explicable on other grounds. There was no purpura or hyperbilirubinemia, and no abnormal neurologic physical signs were found. It is

9. Zeek, P. M.; Smith, C. C., and Weeter, J. C.: Studies on Periarteritis Nodosa: III. The Differentiation Between the Vascular Lesions of Periarteritis Nodosa and of Hypersensitivity, Am. J. Path. **24**:889-917 (July) 1948.

10. Symmers, W. St. C.: Necrotizing Pulmonary Arteriopathy Associated with Pulmonary Hypertension, J. Clin. Path., to be published.

11. P. W. C. [P. W. Clough?]: Thrombotic Thrombocytopenic Purpura, Editorial, Ann. Int. Med. **33**:739-743 (Sept.) 1950.

entirely hypothetical whether convincing evidence of this disease might have been manifested had the patient survived longer.

As the finding of a hemorrhagic lesion involving one of the mammillary bodies had suggested that the mental disorder observed during the last weeks of life might have been similar in origin to the psychosis of Wernicke's encephalopathy, it is noteworthy that histologic examination showed none of the characteristics of the latter.[12] In view of the intracerebral platelet thromboses it is of interest that hemorrhagic encephalopathy due to platelet thrombosis has been recorded.[13]

### Disseminated Lupus Erythematosus

A relationship between the platelet thrombosis syndrome and the so-called collagen diseases has been suggested.[14] Contrary views have been expressed,[15] although disseminated lupus erythematosus and polyarteritis nodosa have each been reported in siblings of patients with the platelet thrombosis syndrome.[16]

In view of these observations it is of special interest that platelet thrombi were present in many vessels in the case of polyarteritis nodosa under discussion and that certain histologic features were present which exactly reproduced the changes in the kidneys, the spleen, and the arterioles in disseminated lupus erythematosus. We are uncertain what interpretation to put on the presence of the latter features, particularly the fibrinoid vascular lesions; they seem to indicate the existence of an alteration of collagen such as occurs in disseminated lupus erythematosus. No focal lesions were found in the subserosal or retroperitoneal fibrous tissue, nor were the peculiar "hematoxylin-staining bodies" described by Klemperer and his colleagues [17] seen.

Klemperer, Pollack, and Baehr [18] considered the periarteriolar sclerosis in the spleen to be a specific characteristic of disseminated lupus erythematosus. Identical "onion peel" sclerosis around the central arterioles may, however, be seen in sarcoidosis [19] and possibly as a result of reactive fibrosis around thrombosed follicular

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12. Campbell, A. C. P., and Biggart, J. H.: Wernicke's Encephalopathy (Polioencephalitis Haemorrhagica Superior): Its Alcoholic and Non-Alcoholic Incidence, J. Path. & Bact. **48**:245-262 (March) 1939.

13. Wyatt, J. P., and Lee, R. S.: Hemorrhagic Encephalopathy Due to Disseminated Thrombocytic Thrombosis: Report of a Case, Arch. Path. **49**:582-589 (May) 1950.

14. Beigelman, P. M.: Variants of the Platelet Thrombosis Syndrome and Their Relationship to Disseminated Lupus, A. M. A. Arch. Path. **51**:213-223 (Feb.) 1951. Symmers, W. St. C., and Barrowcliff, D. F.: Platelet Thrombosis Syndrome, Proc. Path. Soc. Great Britain & Ireland, July 1951, J. Path. & Bact. **63**:552-554 (July) 1951.

15. Singer, K.; Bornstein, F. P., and Wile, S. A.: Thrombotic Thrombocytopenic Purpura: Hemorrhagic Diathesis with Generalized Platelet Thromboses, Blood **2**:542-554 (Nov.) 1947. Klemperer, P.: Proc. Am. A. Path. & Bact., March 1948, Am. J. Path. **24**:704 (May) 1948.

16. Singer, K.; Motulsky, A. G., and Shanberge, J. N.: Thrombotic Thrombocytopenic Purpura: II. Studies on the Hemolytic Syndrome in This Disease, Blood **5**:434-448 (May) 1950.

17. Klemperer, P.; Gueft, B.; Lee, S. L.; Leuchtenberger, C., and Pollister, A. W.: Cytochemical Changes of Acute Lupus Erythematosus, Arch. Path. **49**:503-516 (May) 1950.

18. Klemperer, P.; Pollack, A. D., and Baehr, G.: Pathology of Disseminated Lupus Erythematosus, Arch. Path. **32**:569-631 (Oct.) 1941.

19. Teilum, G.: Allergic Hyperglobulinosis and Hyalinosis (Paramyloidosis) in the Reticulo-Endothelial System in Boeck's Sarcoid and Other Conditions: A Morphologic Immunity Reaction, Am. J. Path. **24**:389-407 (March) 1948.

arterioles in cases of platelet thrombosis.[20] In relation to the last observation it may be remarked that in our case the vessels which showed concentric sclerosis were not affected by thrombosis.

It is not suggested that disseminated lupus erythematosus was present in this case; the histologic picture was incomplete, there was no skin disease, and no endocarditis was found. The case is important, however, because it presented the coexistence of lesions characteristic of three distinct varieties of "collagen disease": polyarteritis nodosa, lupus erythematosus, and platelet thrombosis.

### Relationship of the Polyarteritis to the Pulmonary Tuberculosis and the Sarcoidosis-like Lymphadenopathy

The coexistence of polyarteritis nodosa and active tuberculosis seems to have been described only once.[21] In view of the prevalence of tuberculosis it is surprising that these diseases have not, apparently, been observed together oftener. In our case there is not sufficient evidence to justify conclusions concerning a possible etiologic relationship between the acutely reactivated pulmonary tuberculosis and the vascular lesions.

Bacteriologically proved pulmonary tuberculosis was associated in this case with a generalized lymphadenopathy in which the normal structure was obliterated by a sclerotic granulomatous process which morphologically could not be distinguished from the late sclerotic stage of sarcoidosis. No lesions resembling sarcoidosis were found in any other organs or tissues, and the only other evidence in favor of sarcoidosis was hyperglobulinemia, the significance of which in the presence of polyarteritis nodosa is equivocal. It must, therefore, be stressed that the strict diagnostic criteria of sarcoidosis [4] were not present; moreover, the observation that the sclerosing granulomatosis was limited to the lymph nodes is contrary to sarcoidosis, in which other tissues are usually demonstrably involved.

The nature of sarcoidosis remains controversial, although a tuberculous causation is much favored.[22] In the case under discussion it is noteworthy that the acute, necrotizing tuberculous pneumonia, with remarkably little defensive tissue reaction, was obviously a recent complication of the much older, fibrocaseous and silicotuberculous lesions. It may be deduced that until quite recently the patient had presented high resistance to the infection. It is impossible to estimate the relative ages of the chronic pulmonary tuberculosis and the sarcoidosis-like lymphadenopathy, but the latter was obviously of much greater age than the acute tuberculous pneumonia. It has been said that caseating tuberculosis develops in at least 10% of patients with sarcoidosis [22b]; it is usually accompanied with recession of the sarcoid lesions, or these are transformed into "banal" tuberculous tissue, although occasionally lesions of sarcoidosis, particularly those in the skin, may coexist with active pulmonary tuberculosis.[22a]

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20. Gore, I.: Disseminated Arteriolar and Capillary Platelet Thrombosis: A Morphologic Study of Its Histogenesis, Am. J. Path. **26**:155-175 (Jan.) 1950.

21. Contratto, A. W.: Periarteritis Nodosa: A Report of Two Cases, One with Special Reference to Sensitivity Factors, Arch. Int. Med. **80**:567-578 (Nov.) 1947.

22. (*a*) Leitner, S. J.: Der Morbus Besnier-Boeck-Schaumann: Chronische epitheloidzellige Retikuloendotheliose sive Granulomatose, Basle, Benno Schwabe & Co., 1942, Chap. 4. (*b*) Freiman, D. G.: Sarcoidosis, New England J. Med. **239**:664-671 (Oct. 28); 709-716 (Nov. 4); 743-749 (Nov. 11) 1948.

It is possible that in our case silicosis may have determined the development of frank tuberculosis in the lungs of a patient whose reaction to the tubercle bacillus might otherwise have been limited to a pure sarcoidosis-type response. We know of no recorded observations of concomitant silicosis and sarcoidosis. No lymph node silicosis was found in our case.

As far as we are aware, there are no published accounts of an association of sarcoidosis and polyarteritis nodosa, lupus erythematosus, or platelet thrombosis. It is curious that a generalized lymphadenopathy with the histologic picture of sarcoidosis should occur in a patient with polyarteritis nodosa; the latter is widely believed to be a manifestation of allergic hypersensitization, whereas sarcoidosis has been thought to represent the reaction to infection of a patient who has a high degree of immunity and no associated hypersensitivity or, alternatively stated, whose tissues present a state of "positive anergy."[23] At first sight it is somewhat difficult to postulate an etiologic relationship between the tuberculosis and both polyarteritis nodosa and the concomitant sarcoidosis-like affection, if the theories of the respectively "hyperergic" and "anergic" nature of the two latter conditions are valid. The lymphadenopathy, however, did not have the histologic appearances of active, progressing sarcoidosis, but had reached the advanced, sclerotic stage and seemed to be almost "burnt out"; it was obviously of much greater age than the arteritis. If, therefore, the polyarteritic and sarcoidosis-like lesions are considered as morphologic manifestations of opposite and noncontemporaneous phases of allergic reactivity to one and the same infection, it seems to be possible that both conditions had a common fundamental cause: the presence of tuberculous infection.

It is noteworthy that the patient had had no contact with any substances, such as sulfonamides and antiserums, which it is recognized may be associated with the development of polyarteritis nodosa. Similarly, there was no evidence of any of the animate or inanimate agents which may provoke lesions mimicking sarcoidosis.[22b]

## SUMMARY

A case of necrotizing arteritis and arteriolitis is recorded in which multiple large aneurysms were present on visceral arteries, death resulting when an aneurysm perforated into the duodenum. Histologically these lesions had the characteristic appearances of polyarteritis nodosa.

Clinical evidence of arterial hypertension was reflected microscopically in widespread hypertensive arteriolosclerosis and in the presence of histologic evidence of malignant hypertension.

Certain changes which are usually associated with disseminated lupus erythematosus were also present, although there were no cardiac lesions and no history or evidence of dermatosis.

Platelet thrombosis affected many smallest-caliber blood vessels. Unequivocal manifestations of the platelet thrombosis syndrome were not noted during life.

Nodular pulmonary silicosis was associated with chronic, fibrocaseous pulmonary tuberculosis; acute, necrotizing tuberculous pneumonia had developed.

A generalized lymphadenopathy presented lesions histologically identical with the late sclerotic stage of sarcoidosis; no other lesions of this type were found.

An attempt is made to correlate and explain the significance of the diverse pathologic findings.

23. Jadassohn, J.: Die allgemeine Pathologie der Tuberkulide, Wien. med. Wchnschr. **64**:36-38, 1914.

# THROMBOEMBOLIC PULMONARY ARTERITIS AND VASCULAR SCLEROSIS

## Its Experimental Production in Rabbits by Means of Intravenously Injected Human Amniotic Fluid and Autogenous Blood Clots

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AND
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THERE are increasing indications in the literature that the widespread presence of small emboli or thrombi within the pulmonary arterial tree may be associated with the development of pulmonary vascular sclerosis. In a separate report [1] we have presented two additional human cases in which this association was observed and have reviewed the available evidence supporting a cause-and-effect relationship between the presence of occlusive masses within the pulmonary arteries and the vascular sclerosis (pulmonary arteriosclerosis). This vascular sclerosis in the human has been attended by the clinical picture of cardiopulmonary failure (Ayerza's syndrome).

In the present communication we wish to bring forward additional experimental evidence supporting the view that occlusive masses within the small pulmonary arteries evoke pulmonary vascular sclerosis. The embolic approach has been used. Periodic intravenous injections of two suspensions, one of human amniotic fluid and the other of fresh autogenous blood clots, have been made in rabbits. The results are considered to extend the observations of C. V. Harrison,[2] who encountered arteritis and transient endarteritis in rabbits following the intravenous injection of human fibrinous emboli. Since these studies were completed, we have learned, through the courtesy of Dr. Howard T. Karsner, that Wartman, Hudson, and Jennings [3] have conducted similar experiments. Although our results are comparable to those of Wartman and co-workers, there appear to be sufficient differences in the two studies to warrant publication of this material.

### METHODS

Adult white rabbits of either sex, weighing approximately 2 kg., were used. They were fed a commercial preparation [4] and water ad libitum.

From the Department of Pathology, Southwestern Medical School of the University of Texas.

1. Muirhead, E. E.; Montgómery, P. O'B., and Gordon, C. E.: Thrombo-Embolic Pulmonary Vascular Sclerosis: 1. A Case Following Pregnancy. 2. A Case Associated with Cryoglobulinemia, to be published.

2. Harrison, C. V.: Experimental Pulmonary Arteriosclerosis, J. Path. & Bact. **60**:289-293, 1948.

3. Wartman, W. B.; Hudson, B., and Jennings, R. B.: (*a*) Experimental Arterial Disease: I. The Reaction of the Pulmonary Artery to Minute Emboli of Blood Clot; to be published; (*b*) II. The Reaction of the Pulmonary Artery to Emboli of Filter Paper Fibers, to be published.

4. The diet consisted of purina® pellets, made by the Purina Ralston Company, St. Louis.

Group 1 (human amniotic fluid). Twelve rabbits received intravenous injections of human amniotic fluid. The fluid was obtained under sterile precautions in the delivery room and stored at 3 F. Cultures of the fluid on blood agar plates were sterile. The fluid was injected as a 1:2 suspension in sterile sodium chloride solution, 1 to 2 cc. being injected every 7 to 10 days. Eleven animals died after one to two injections. One animal, the 12th, received six injections over a period of 57 days. This animal died seven days after the last injection.

Group 2 (autogenous blood clots). Thirteen rabbits received intravenous injections of their own blood clots. On each occasion, the animal was bled from the marginal vein of the ear into a sterile Petri dish, and the blood was allowed to clot while the dish was slanted. During the first three weeks 5 drops of blood were used, while thereafter 10 to 15 drops were used. The whole clot was cut into fragments less than 0.5 mm. in diameter by means of rapidly criss-crossing sterile razor blades. This procedure was usually begun within 10 to 15 minutes after the blood was shed. The small fragments of clot were mixed with 5 cc. of sterile saline and sucked into a syringe through a 19-gauge needle. The entire amount was injected into an ear vein of the same rabbit through the 19-gauge needle. The injection was conducted over a period of two to three minutes. It was estimated that approximately 80% of the clotted blood was reinjected in this manner.

Five rabbits died within one to two weeks after one or two injections. Eight rabbits survived over a period varying between 35 and 95 days; most of this group was killed. In the latter group, a rabbit (No. 8) succumbed on the 35th day after six injections at seven-day intervals. Five rabbits (Nos. 9 to 13) were killed on the 41st day after six injections at seven-day intervals.

TABLE 1.—*Incidence of Various Pulmonary Lesions Encountered in the Twelve Rabbits Receiving Human Amniotic Fluid Intravenously*

| Pulmonary Lesion | Rabbits (Total 12) |
|---|---|
| 1. Edema | 6 |
| 2. Focal hemorrhages | 7 |
| 3. Focal acute granulomatous pneumonitis | 10 |
| 4. Fibrinous thrombi | 7 |
| 5. Acute arteritis | 7 |
| 6. Vascular sclerosis (endarteritis mainly) | 4 |

One rabbit (No. 7), which received five injections at 10-day intervals, was killed 20 days after the last injection. One rabbit (No. 5), which received one injection weekly for 75 days, was killed 20 days after the last injection.

At autopsy, multiple sections were taken from each lobe of the lungs. In addition, sections were taken from the heart, the liver, the kidneys, and the adrenals. The sections were prepared in the usual manner after formalin fixation. The hematoxylin-eosin and elastic tissue (Verhoeff-Van Gieson) stains were used on all lung sections. In addition, typical examples from each group were stained with Masson's trichrome stain (Goldner), phosphotungstic acid-hematoxylin stain, and Mallory's connective tissue stain.

As controls, 20 rabbits from the same colony, killed for other purposes, were used. The pulmonary vessels of these animals were normal. In four rabbits, near blood vessels there were microscopic granulomas consisting of epithelioid cells, giant cells, and a mantle of lymphocytes. These lesions apparently were similar to those observed by Wartman, Hudson, and Jennings [3] in their control group.

## RESULTS

*Group 1* (amniotic fluid).—Table 1 relates the major microscopic findings in the lungs. Eight rabbits died after the first injection. Three rabbits died after the second injection, having survived into the second week. One rabbit survived 64 days, after six injections.

As listed in Table 1, the predominant lesions were of an acute type. Embolization by lanugo hairs and squama, as described originally by Steiner and

Lushbaugh,[5] was noted. Pulmonary edema and focal hemorrhages were observed in over 50% of the animals. The hemorrhages had the gross appearance of infarcts, but microscopically necrosis was not noted. There were additional striking vascular and parenchymal lesions. Numerous fibrin thrombi, contained within small arteries and arterioles, as described by Schneider,[6] were observed in seven animals. An acute granulomatous pneumonitis of focal distribution was readily detected in 10 animals. These foci could be identified, in some sections, as originating about embolic masses in small blood vessels. Squama represented the common foreign substance, about which usually the vessel wall was severely damaged. Giant cells, macrophages, and lymphocytes were noted within the lumen and in the vessel wall and its environs. In other areas the parenchyma was overrun by inflammatory cells; thus a possible vascular origin was obscured. These foci usually covered a lower-power field of $\times$ 50 size. The main inflammatory cells were giant cells and macrophages, which together in some zones assumed a tuberculoid appearance. In addition, lymphocytes and neutrophils were present in varying numbers. Eosinophils were absent. The focal pneumonitis frequently occurred near a bronchiole, the cellular exudate partly surrounding the bronchiole and giving the picture of peribronchiolitis. The later type of granuloma, which did not appear to be related to embolic masses, resembled the granuloma encountered by Wartman, Hudson, and Jennings,[3] in their control series and seen also in 4 of our 20 control rabbits. Thus, two types of granulomas were observed, one similar to that seen in the control animals and obviously of no significance in these studies, and a second type which appeared to be a granulomatous arteritis, apparently a foreign body response due to the intravascular presence of squamae and lanugo hairs.

The small pulmonary arteries displayed two forms of injury, one acute, in the form of an acute arteritis, and the other subacute or chronic, in the form of vascular sclerosis. The acute arteritis was common in seven rabbits. It was characterized by damage to all layers of the arterial wall plus a heavy cellular infiltration (Fig. 1*A*). The adventitia revealed edema and was heavily infiltrated with neutrophils, lymphocytes, and cells resembling macrophages. The media was the seat of necrosis, being transformed into the reddish eosinophilic smudge frequently designated as "fibrinoid necrosis." Neutrophils were also prominent in this layer, as well as distorted nuclear remnants. The internal elastic lamina was disrupted. The endothelium was frequently intact, but at times endothelial proliferation was prominent, almost filling the lumen of smaller arteries. This acute arteritis with its neutrophilic exudate resembled periarteritis nodosa as seen in human cases.

The subacute and chronic vascular changes occurred in four rabbits. Three rabbits received two injections, and one rabbit survived 64 days and six injections. The latter animal displayed many of these lesions, whereas in the former they had to be searched for. There were two types of vascular sclerosis. The first type consisted of fibrosis and hyalinization of the entire arterial wall, with or without elastic hyperplasia (Fig. 1*B* and *D*). The fibrosis at times resembled that of healed

5. Steiner, P. E., and Lushbaugh, C. C.: Maternal Pulmonary Embolism by Amniotic Fluid as Cause of Obstetric Shock and Unexpected Deaths in Obstetrics, J. A. M. A. **117**:1245-1254 (Oct. 11) and 1340-1345 (Oct. 18) 1941.

6. Schneider, C. L.: "Fibrin Embolism" (Disseminated Intravascular Coagulation) with Defribination as One of the End Results During Placenta Abruptio, Surg., Gynec. & Obst. **92**:27-34, 1951.

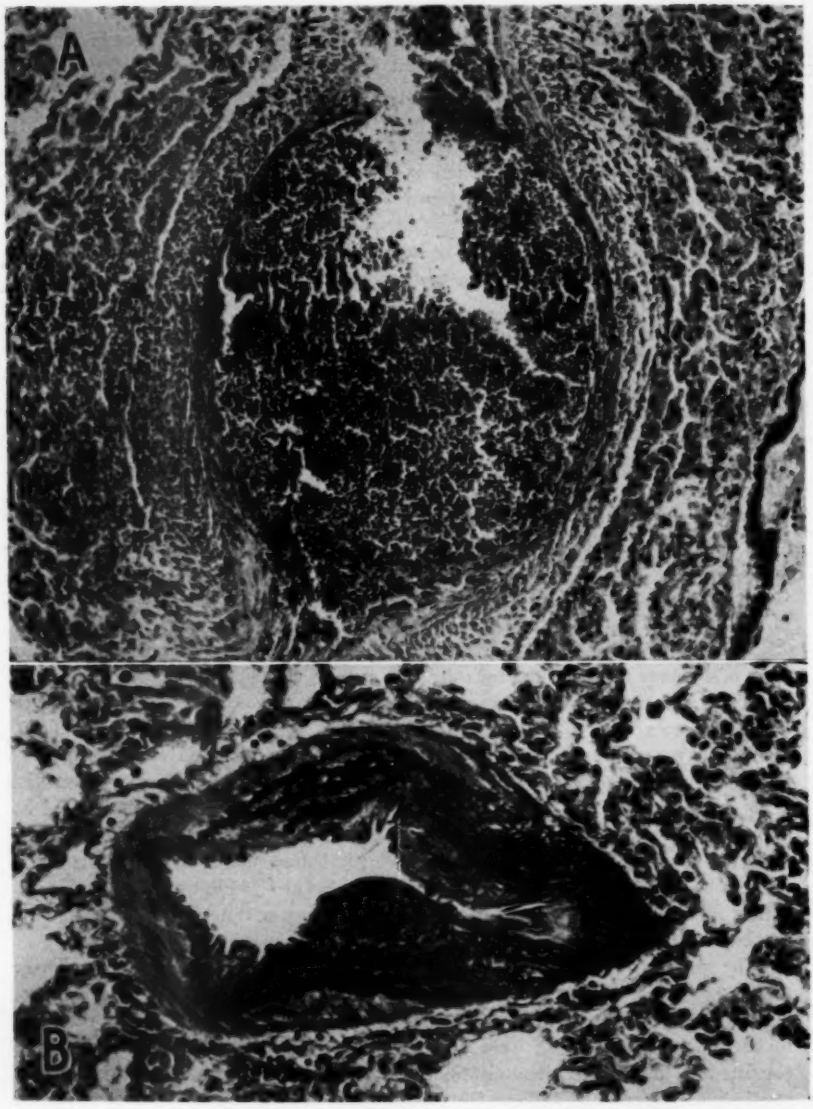
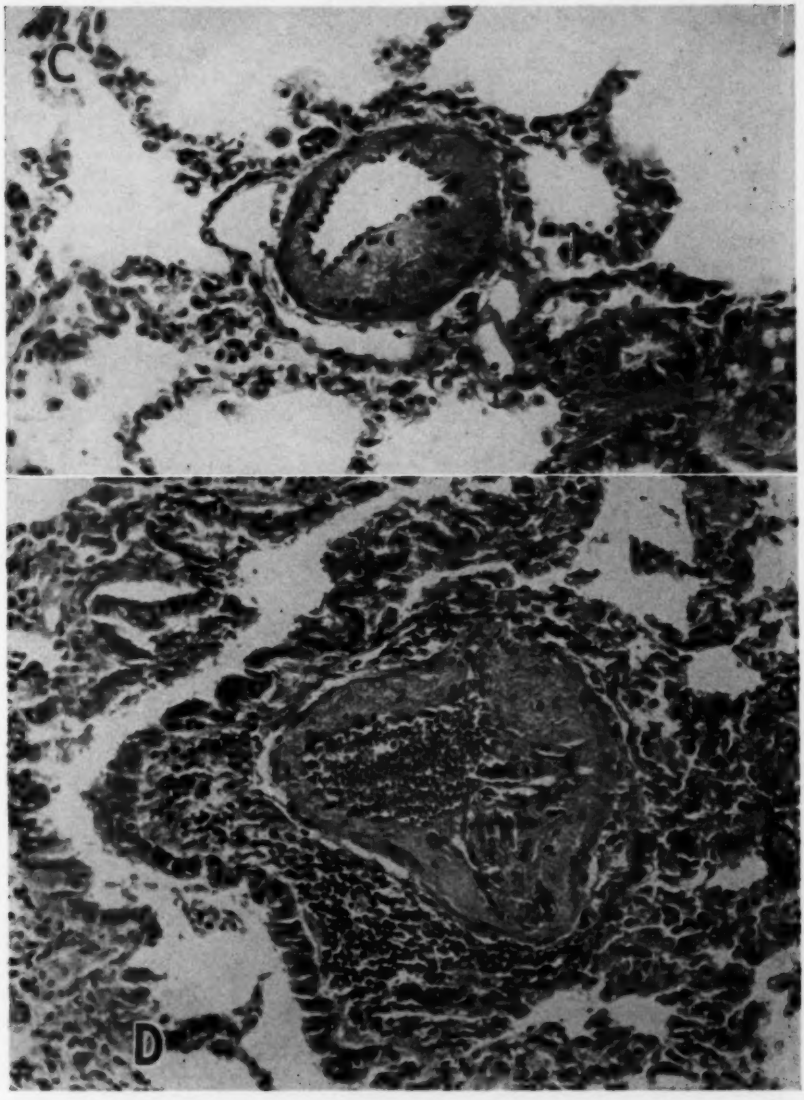



Fig. 1.—*A*, acute arteritis in a rabbit which died after a second injection of human amniotic fluid. The artery shows necrosis of the media and a heavy cellular exudate within the adventitia and the media, the predominant cell being the neutrophil. Rabbit *2*; hematoxylin and eosin; × 140.

*B*, endarteritis due to fibroelastosis of the intima in a rabbit in which death occurred after six injections of human amniotic fluid, 64 days after the first injection. Rabbit 12; elastin stain; × 300.




*C*, hyaline thickening of the intima, with an eccentric position of the lumen and atrophy of the media, in the same rabbit as *B*. Hematoxylin and eosin stain; × 300.

*D*, hyalinization of the arterial wall and moderate endothelial proliferation in the same rabbit as *B*. Hematoxylin and eosin stain; × 210.

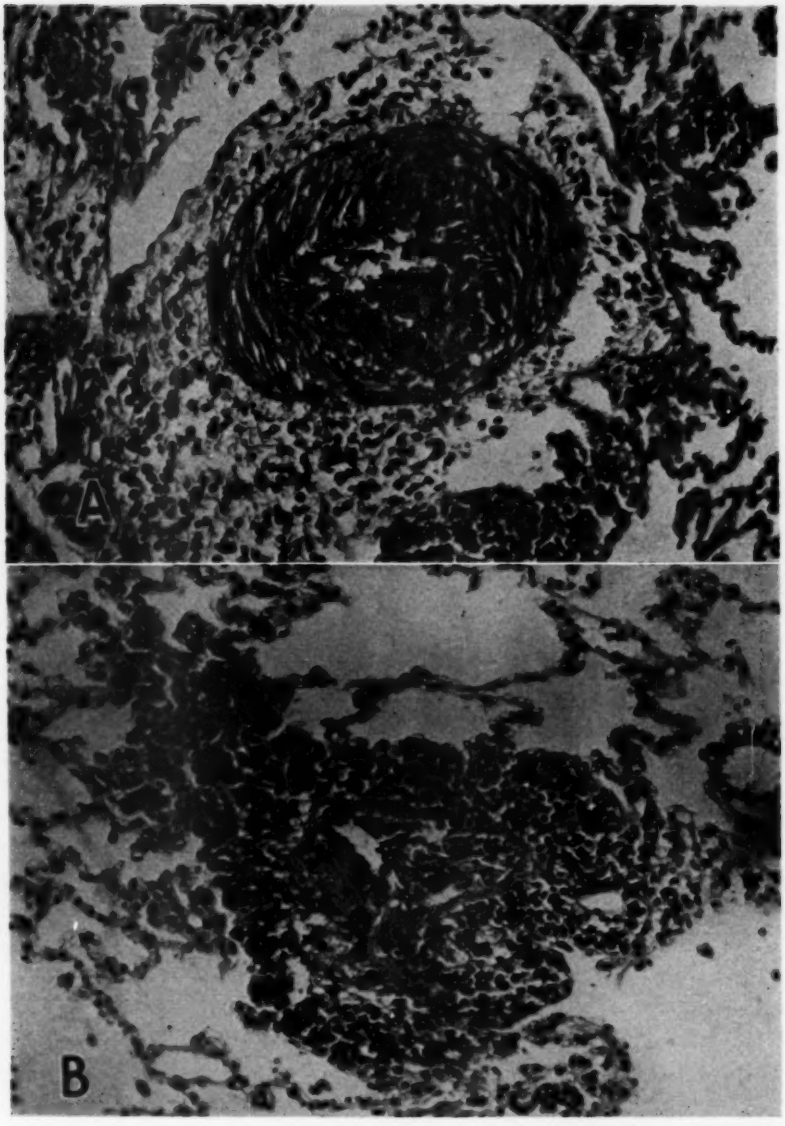
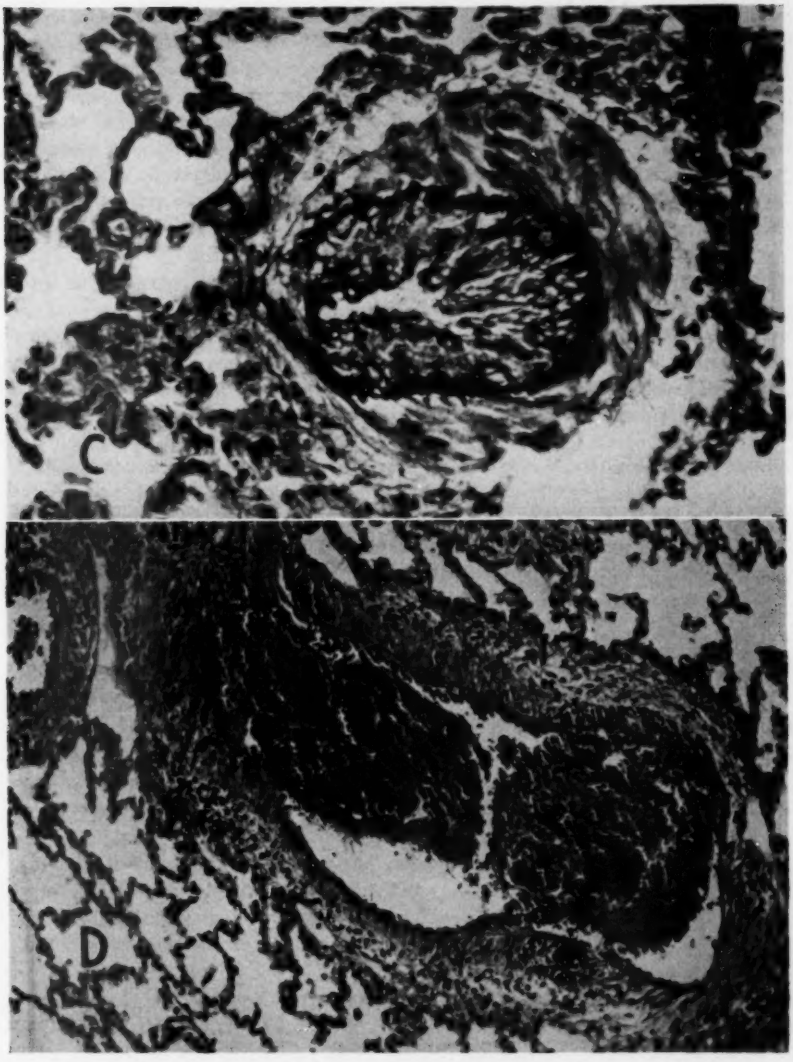



Fig. 2.—*A*, artery showing early endothelial proliferation. The adventitia has a mild cellular exudate, which has extended into the intact media. Rabbit 3, which died after two injections of blood clots; hematoxylin and eosin stain; × 210.

*B*, acute arteritis. The vessel shows proliferation of the endothelium, necrosis of the media, and a heavy exudate in the adventitia, consisting mainly of neutrophils and lymphocytes. Rabbit 4, which died after two injections of blood clots; hematoxylin and eosin stain; × 250.




*C*, endarteritis obliterans. The artery shows a small, eccentric lumen. The internal elastic lamina is well defined and the loose structure of the connective tissue in the intima is shown. Rabbit 5, which died after 95 days and six injections of blood clots; elastin stain; × 380.

*D*, acute arteritis associated with a blood clot in the lumen. Edema of the adventitia and necrosis of the media are shown. The cellular exudate is mostly in the adventitia. Rabbit 3, in which death followed the second injection of blood clots; hematoxylin and eosin stain; × 140.

arteritis and was so interpreted. The second form of sclerosis (Fig. 1*B* and *C*) consisted mainly of thickening of the intima associated with narrowing of the lumen (endarteritis). Elastosis of the intima was also noted (Fig. 1*B*). The intimal thickening, when pronounced, was associated with atrophy of the media (Fig. 1*C*).

Although these acute and chronic arterial lesions were readily demonstrated, the sections demonstrated many unaltered arteries. Significance is being attached to the mere development of these lesions, but it is well recognized that the over-all functional impairment eventuating from their presence could not be evaluated by their morphologic demonstration. It seems apparent from the results that the procedure was highly lethal, which in itself suggests an important functional position of the lesions.

*Group 2* (embolism by autogenous blood clots).—The pulmonary lesions associated with the injection of autogenous fresh blood clots were similar to those following the injection of human amniotic fluid. As noted in Table 2, acute and subacute or chronic lesions were observed. Pulmonary edema was not as common as intra-alveolar capillary hemorrhages. Scattered pulmonary infarcts were detected in only one animal. The focal pneumonitis occurred in the vicinity of vascular

TABLE 2.—*The Incidence of Various Pulmonary Lesions Encountered in Thirteen Rabbits Receiving Autogenous Blood Clots Intravenously*

| Pulmonary Lesion | Rabbits (Total 13) |
|---|---|
| 1. Edema | 4 |
| 2. Focal hemorrhages | 10 |
| 3. Focal pneumonitis | 4 |
| 4. Emboli (fibrinous mainly) | 5 |
| 5. Endothelial proliferation | 10 |
| 6. Acute arteritis | 11 |
| 7. Vascular sclerosis | 7 |

lesions, usually of the acute type. The inflammatory cells, neutrophils, lymphocytes, and macrophages, were mostly interstitial in location. On occasions the cellular exudate extended to the wall of bronchioles, effecting a peribronchiolitis. One animal displayed interstitial granulomatous foci consisting mainly of macrophages heavily laden with hemosiderin.

The vascular lesions were widespread, being observed within most sections, taken from all lobes. However, many intact arterial segments remained. Eleven animals displayed acute arterial lesions. There were two significant changes in the arterial wall, namely, endothelial proliferation and acute necrotizing arteritis. These two alterations occurred singly but frequently were concomitant. The endothelial proliferation was disorderly, consisting of an irregular piling up of these cells toward the lumen (Figs. 2*A* and *B* and 3*A* and *C*). Circumferential endothelial proliferation, as well as focal proliferation forming buds projecting into the lumen, were noted. It is of interest that the endothelial proliferation occurred in vessels whose lumen was free of occlusive masses. Emboli, which were noted in five animals, had a fibrinous appearance and at times were invaded by endothelial cells. The acute arteritis, observed in 11 animals, was identical with that described as following the injection of human amniotic fluid. The adventitia was edematous and heavily infiltrated with cells, neutrophils and lymphocytes mainly (Fig. 2*A*, *B*, and *D*).

The media was necrotic and contained distorted nuclei, neutrophils and lymphocytes. The endothelium was frequently active; at times the lumen was blocked by masses of endothelial cells. The subacute or chronic vascular lesions (vascular sclerosis), just as in Group 1, consisted of diffuse fibrosis and hyalinization of the vessel wall (Figs. 2*B* and 3*B*) and fibrous thickening of the intima (endarteritis). The former lesion on occasions was associated with the development of aneurysmal dilatation of the arterial wall (Fig. 3*B*). Endarteritis was the most common lesion. In this group elastosis of the intima was rare, the main lesion being characterized by a narrowed lumen, frequently eccentric, owing to the accumulation of cells and a loose fibrillar intercellular material between the intact internal elastic lamina and the intact endothelium (Figs. 2*C* and 3*A*, *C* and *D*). There appeared to be a transition between endothelial proliferation, the presence of endothelial cells and fine fibrils, and the development of a more mature type of intercellular substance within the intima. The media of these arteries was usually intact, at times displaying evidence of atrophy (Fig. 3*C* and *D*). The connective tissue stains (Masson's trichrome, phosphotungstic acid-hematoxylin, and Mallory's stain) supported the interpretation that the material in the intima and the media of the sclerotic vessels represented scars of various ages.

These observations demonstrated a similarity between the pulmonary vascular lesions occurring in rabbits subsequent to intravenous injections of human amniotic fluid and autogenous fresh whole blood clots. The acute vascular lesion consisted of an acute arteritis resembling periarteritis nodosa, which was associated with endothelial proliferation. The subacute or chronic lesion consisted of arterial sclerosis characterized mainly by endarteritis obliterans but also displaying fibrosis and hyalinization of the entire arterial wall.

## COMMENT

The observations of C. V. Harrison [2] dealing with pulmonary arterial lesions associated with emboli that had been intravenously injected into rabbits have been extended. Harrison injected white emboli, consisting mainly of fibrin obtained by washing clotted human blood relatively free of the cellular elements. In the herein-reported observations fresh whole blood clots were cut into small fragments and injected into the same animal. Whereas Harrison noted an acute arteritis and a transient endarteritis consisting mainly of intimal elastosis, we have observed an acute arteritis with a prominent neutrophilic exudate resembling periarteritis nodosa and a vascular sclerosis having the characteristics of a permanent lesion. Thus, modified results of the same basic process have been obtained. In addition, similar lesions were obtained by repeated injections of small quantities of human amniotic fluid. Wartman, Hudson, and Jennings [3a] likewise injected autogenous clots into rabbits and noted endothelial hyperplasia, adherent emboli, and focal fibrous intimal or medial scars.

The arterial sclerosis was widespread in the terminal portions of the pulmonary arterial tree and resembled the lesions of the human described as primary pulmonary vascular sclerosis. There were three main changes in these small arteries: (1) endothelial proliferation, (2) fibrosis and hyalinization of the entire wall, and (3) endarteritis obliterans. The endothelial proliferation occurred either by itself or in conjunction with the acute arteritis. At times the entire lumen was filled with an

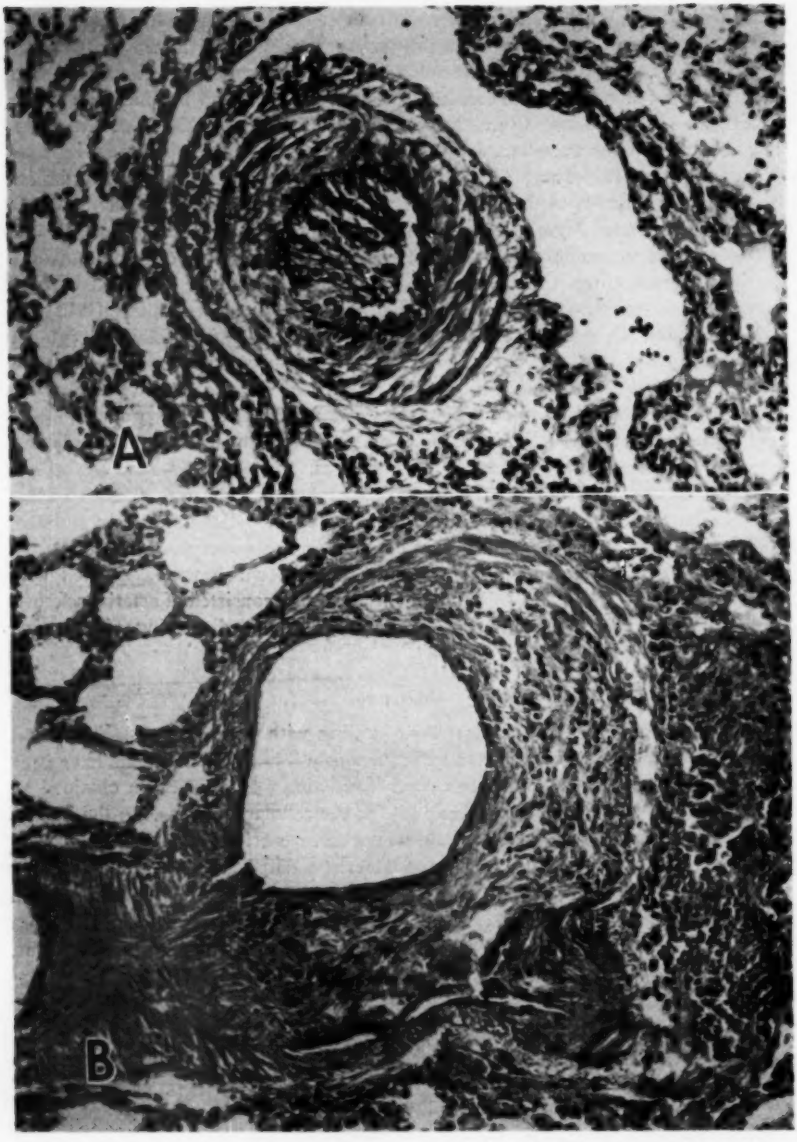
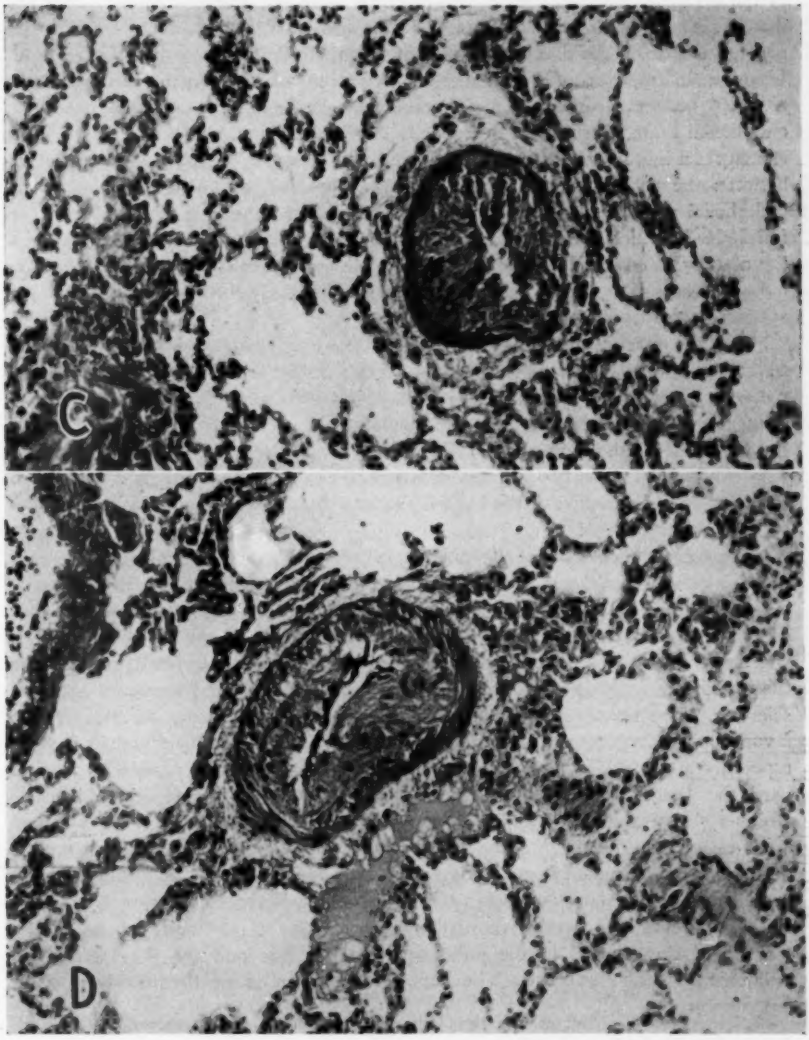



Fig. 3.—*A*, endarteritis obliterans. The lumen is not only narrowed but much distorted, a disturbance explained by the irregular thickening of the intima, which appears to be in keeping with the earlier irregular proliferation of endothelial cells. Rabbit 8, which was killed after 41 days and five injections of blood clots; elastic stain; × 190.

*B*, healed arteritis. The arterial wall is thickened, replaced by a young scar, and displays an aneurysmal bulge. Remnants of the cellular exudate remain in the adventitia. Rabbit 12, killed after 41 days and six injections of blood clots; hematoxylin and eosin stain; × 190.




*C*, endarteritis obliterans. The vessel shows an irregularly narrowed lumen, atrophy of the media, and presence of many plump cells among the connective tissue fibers in the intima. Rabbit 10, killed after 41 days and five injections of blood clots; hematoxylin and eosin stain; × 210.

*D*, endarteritis obliterans with hyalinization of the connective tissue in the intima and atrophy of the media. Rabbit 12, killed after 41 days and six injections of blood clots; hematoxylin and eosin stain; × 210.

irregular accumulation of endothelial cells. The diffuse fibrosis and hyalinization of the arterial wall resembled healed arteritis. The endarteritis obliterans was the most common disturbance. Elastosis of the intima was uncommon, reaching its greatest degree in the one rabbit receiving amniotic fluid over a prolonged period. Usually the endarteritis caused marked narrowing of the lumen, which at times assumed an eccentric location. The internal elastic lamina and the immediate endothelial lining were intact in the fully developed lesion, and between these two structures a loose connective tissue accumulated. There appeared to be a transition between the endothelial proliferation, the laying-down of fine fibrils among the endothelial cells, and an increase in the intercellular substance giving rise to the full-fledged picture of endarteritis obliterans. Thus, the impression was gained that the activity of endothelial cells was responsible for the endarteritis. The media of most of the vessels displaying endarteritis was intact, but on occasions this layer appeared attenuated and atrophic.

The resemblance of the acute arteritis to periarteritis nodosa constituted a striking finding. This is of particular interest, since the lesion evolved in rabbits receiving their own proteins in the form of freshly clotted blood and its formed elements. Since the lesions following the injections of amniotic fluid and blood clots were identical, it appears that the mechanisms of their production were similar. The similarity in the two groups also minimizes the possibility that the lesion resulting from the action of the human amniotic fluid is one produced by a heterospecific substance.

The terms "thrombo-embolic pulmonary arteritis" and "thrombo-embolic pulmonary vascular sclerosis (or endarteritis)" are proposed for the lesions noted. These terms are inclusive, since they consider the possibility of thrombosis secondary to embolism and also allow for the possibility of thrombosis per se, as well as embolism, being responsible for such lesions. There is need to allow for the possibility of widespread pulmonary thrombosis evoking pulmonary vascular sclerosis, since human cases have been described in which this association was favored. The reports of Ljungdahl,[7] Tschistovitsch,[8] and Frothingham[9] were especially striking. Tschistovitsch was so impressed with this possibility that he used the term "chronic pulmonary thrombo-endarteritis" to describe his case. Brenner[10] agreed with the interpretations by considering that thrombosis explained a few cases of so-called primary pulmonary vascular sclerosis. In a separate report, dealing with two human cases, we have reviewed the association of thrombosis and embolism and pulmonary vascular sclerosis, mainly of the endarteritic type.

The observations herein recorded merely indicate a relationship between the lodging of tiny emboli in the pulmonary arterial tree and the development of pulmonary vascular sclerosis. The important question of whether the sclerosis is

7. Ljungdahl, M.: Gift es eine chronische Embolisierung der Lungenarterie? Deutsches Arch. f. klin. Med. **160**:1-23, 1928.

8. Tschistovitsch, T.: Thrombo-endoartérite pulmonaire chronique, Compt. rend. Soc. biol. **89**:627, 1923.

9. Frothingham, C.: A Case of Extensive Bilateral Progressive Thrombosis of the Smaller Branches of the Pulmonary Arteries, Am. J. Path. **5**:11-22, 1929.

10. Brenner, O.: Pathology of the Vessels of the Pulmonary Circulation, Arch. Int. Med. **56**:211-237 (Aug.); 457-497 (Sept.); 724-752 (Oct.); 976-1014 (Nov.); 1189-1241 (Dec.), 1935.

mainly preembolic or postembolic was not elucidated. The mechanisms whereby such emboli effect the sclerosis requires future scrutiny. There is the possibility that the nourishment of the arterial tree is altered by the embolic masses. Such a view would consider the arteritis as a consequence of acute ischemia of the arterial wall followed by necrosis, and the fibrosis and endarteritis as a result of less marked and more prolonged nutritional disturbances. This hypothesis is supported by the view expressed by Brenner,[10] which considers that the vasa vasorum supply the adventitia and the outer portions of the media of small pulmonary arteries, whereas the inner portions of these vessels are nourished directly from the lumen. Conceivably, any interference with the luminal blood flow would alter the source of nourishment for varying distances beyond and before the occlusive mass. From a theoretical point of view, the concept that injury followed by repair effects sclerosis appears as tenable as the often expressed view that pulmonary hypertension per se is the causative factor. To be sure, pulmonary hypertension and pulmonary vascular sclerosis appear to have a close association, but the hypertension can be as readily considered a by-product of the sclerosis, since the right ventricle continues to pump a similar volume of blood into a narrowed arterial bed.

## SUMMARY

Human amniotic fluid and autogenous blood clots injected into rabbits intravenously have been demonstrated to cause widespread acute and subacute or chronic lesions in small pulmonary arteries. The acute lesions consisted of necrotizing arteritis. The subacute or chronic lesions were characterized by fibrosis or hyalinization of the arterial wall and endarteritis obliterans. The latter lesions were observed following a prolonged period of multiple injections lasting 35 to 95 days. These observations are considered to represent extensions of the work of Harrison and Wartman, Hudson, and Jennings.

The pulmonary vascular sclerosis resembled the lesions observed in primary pulmonary vascular sclerosis of the human. The association of thrombosis or embolism and pulmonary vascular sclerosis has been described on several occasions in human cases.

The fundamental cause of the vascular sclerosis was not elucidated. It is postulated that injury to the arterial wall due to nutritional disturbances followed by the working of the repair process as it affects blood vessels appears as plausible a hypothesis for explaining the pathogenesis of the sclerosis as the often expressed view that pulmonary hypertension is the causative factor in widespread pulmonary vascular sclerosis.

# METASTASIS TO THE PINEAL BODY

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METASTASIS to the pineal body, to judge from the literature, is rare. Warren,[1] in 1936, referred to three previous reports and presented his observations in two additional cases. We have been able to find 11 other cases, bringing the total to 16.[2]

Table 1 summarizes the cases in which a cancer metastasized to the pineal body and indicates the incomplete nature of the data that are available. Seven patients were males and nine were females. The primary neoplasm arose in the breast in six, was probably bronchogenic in three, and was a melanoma in three. Of the eight whose cases are adequately described, all had widespread visceral metastases, and all but one had other intracranial metastases as well as involvement of the pineal body. The pineal body itself was usually described as normal in size or slightly enlarged; the largest mass, 1.5 in. (38 mm.) in diameter, was reported by Church.[2d]

The case reported by Jacobi [2g] requires special mention in that it represents the only example in the literature of a metastatic tumor involving the pineal body in

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1. Warren, S.: Studies on Tumor Metastases: VII. Metastasis to the Pineal Gland, Am. J. Cancer **28**:713-719, 1936.

2. (*a*) Askanazy, M.: Die Zirbel und ihre Tumoren in ihrem funktionellen Einfluss, Frankfurt. Ztschr. Path. **24**:58-77, 1920. (*b*) Berblinger, W.: Die Glandula pinealis, in Henke, F., and Lubarsch, O., editors: Handbuch der speziellen pathologischen Anatomie und Histologie, Vol. 8, Drüsen mit innerer Sekretion, Berlin, Julius Springer, 1926, pp. 681-759. (*c*) die Biasi, W.: Ueber Krebsmetastasen in der Milz, Arch. path. Anat. **261**:885-918, 1926. (*d*) Church: Cancer of the Dura Mater and Calvaria with Similar Disease Scattered Through Most of the Organs of the Body, Tr. Path. Soc. London **20**:325-330, 1869. (*e*) Forster: Ein Fall von Markschwamm mit ungewöhnlich vielfacher metastatischer Verbreitung, Arch. path. Anat. **13**:271-274, 1858. (*f*) Gierke, E. von: Hypophysis und Epiphysis bei Diabetes insipidus, Verhandl. deutsch. path. Gesellsch. **25**:200-208, 1914. (*g*) Jacobi, W.: Beitrag zur Kenntnis der Epiphysentumoren, Deutsche Ztschr. Nervenh. **71**:350-357, 1921. (*h*) Ogle, C.: Sarcoma of Pineal Body, Tr. Path. Soc. London **50**:4-6, 1899. (*i*) Walter, F.: Weitere Untersuchungen zur Pathologie und Physiologie der Zirbeldruse, Ztschr. ges. Neurol. u. Psychiat. **83**:411-463, 1923.

which clinical signs of a pineal tumor were present and in which the remainder of the brain failed to reveal neoplastic invasion. Unfortunately, the tumor of the pineal body was described as a "large cell sarcoma" and was not illustrated. The patient had a similar tumor involving the right ovary, the adrenals, the kidneys, lymph nodes and bone, and the possibility of a lymphoma or of two primary tumors cannot be excluded.

Our interest in the question was stimulated by the chance observation of two instances of metastatic involvement of the pineal body. A more systematic survey

TABLE 1.—*Cases of Metastatic Involvement of the Pineal Body in the Literature*

| | Author | Sex | Age, Yr. | Primary Tumor and Metastases | Brain Involvement |
|---|---|---|---|---|---|
| 1. | Forster,[2e] 1858.... | M | 37 | Widespread in abdominal organs, lungs, heart, bones, skin, and left hilum of lung | Extensive in brain and dura |
| 2. | Church,[2d] 1869.... | M | 47 | Widespread in abdominal organs, bones, skin, lungs, and heart | Extensive in dura |
| 3. | Ogle,[2b] 1869..... | F | 32 | Thorax and abdomen stated to be free | Diffuse melanomatosis of dura and underlying cortex |
| 4. | von Gierke,[2f] 1914 | F | 72 | Carcinoma of breast involving lungs, kidneys, and bones | Dural nodules |
| 5. | Askanazy,[2a] 1920. | F | 47 | Carcinoma of breast with recurrence | Brain, meninges, spinal cord, and choroid involved |
| 6. | Ibid. ..... | M | 55 | Left bronchus, liver, pancreas, bones, and adrenals | Brain and choroid |
| 7. | Jacobi,[2g] 1921..... | F | 21 | Sarcoma in right ovary, adrenals, kidneys, lymph nodes, and bone | Confined to pineal body |
| 8. | Walter,[21] 1923.... | F | 54 | Autopsy confined to brain (hypernephroma) | Incomplete description |
| 9. | Berblinger,[2b] 1926. | M | 38 | Squamous cell carcinoma of frontal sinus; data incomplete | Incomplete description |
| 10. | Ibid. ..... | F | 44 | Carcinoma of breast involving thoracic and abdominal organs, bones | Meninges involved |
| 11. | Ibid. ..... | F | 42 | Carcinoma of breast; data incomplete | Incomplete description |
| 12. | Ibid. ..... | M | 65 | Melanoma of foot; data incomplete | Incomplete description |
| 13. | Ibid. ..... | M | 42 | Melanoma of skin; data incomplete | Incomplete description |
| 14. | di Biasi,[2c] 1926... | F | 69 | Carcinoma of breast involving lungs, spleen, kidneys, adrenals, thyroid and bones | Incomplete description |
| 15. | Warren,[1] 1936.... | F | 60 | Carcinoma of breast extremely widespread in thorax and abdomen | Massive growths in brain and meninges |
| 16. | Ibid. ..... | M | .. | Melanoma of thigh; data incomplete | Incomplete description |

of available pathologic material was then undertaken to ascertain the frequency of the finding.

## MATERIALS AND METHODS

Brains from 130 unselected autopsies on patients with metastasizing neoplastic disease were examined. Whenever possible, a consecutive series was sought, especially those cases previous and subsequent to the two initial cases. Brains of patients with lymphomas or leukemias were excluded.

The pineal body was removed, and at first a single section was examined. The brain was carefully examined in the gross, and at least five sections were obtained for microscopic study. In cases of apparently isolated metastasis to the pineal body the brain was reinvestigated and multiple segments were taken for histologic examination. Cases in which there was extensive tumor involvement of the choroid plexus and meninges, with the possibility that such involvement might have extended directly to the pineal body, were not considered as metastasis to the pineal body. The criterion for metastasis to the pineal body was a growth extending from the central portions, frequently with a rim of intact pineal tissue on the periphery.

There were 96 males and 35 females in the series; the preponderance of males was due to a large source of the material from the Veterans Administration Hospital in San Francisco. The ages of the patients varied from 2 to 80 years. The types of neoplastic diseases represented in the series are given in Table 2.

## RESULTS

Of 130 brains from patients with disseminated neoplastic disease, 32, or 25%, showed metastasis. Metastasis was found in the pineal body in five cases, or 4% of the series.

The salient findings in the five cases were as follows:

CASE 1.—T. M., a man, 44, complained of intermittent nausea and vomiting of one year's duration and of severe epigastric pain and the appearance of multiple tender subcutaneous nodules of one month's duration. Physical examination revealed the subcutaneous nodules and generalized lymphadenopathy. There were dulness and decrease in breath sounds over the left superior lung field. The results of a neurologic examination were normal. Roentgenograms of the chest showed an infiltration of the upper lobe of the left lung. A biopsy of a subcutaneous nodule demonstrated an anaplastic carcinoma.

TABLE 2.—*Data from Brains Examined for Presence of Pineal Metastases in Cases of Disseminated Neoplastic Disease*

| Site or Type of Primary Cancer | Cases | Number in Which Brain Was Involved by Tumor | Number in Which Pineal Body Was Involved by Tumor |
|---|---|---|---|
| Lung | 26 | 12 | 3 |
| Breast | 10 | 5 | 2 |
| Kidney, bladder | 9 | 2 | .. |
| Mouth, pharynx | 9 | 1 | .. |
| Stomach | 9 | 2 | .. |
| Melanoma | 9 | 4 | .. |
| Pancreas | 8 | .. | .. |
| Testis | 7 | 1 | .. |
| Uterus | 7 | 1 | .. |
| Liver | 7 | .. | .. |
| Colon | 5 | .. | .. |
| Prostate | 4 | 1 | .. |
| Thymus | 2 | .. | .. |
| Thyroid | 2 | .. | .. |
| Esophagus | 2 | .. | .. |
| Sarcoma | 10 | 2 | .. |
| Miscellaneous * | 4 | .. | .. |
| Total | 130 | 32 | 5 |

* One each of antrum, adrenal, ovary, and chromaffin tissue.

The patient died one month after being admitted to the hospital. At autopsy the peritoneal cavity contained 4 liters of purulent fluid arising from a duodenal perforation. A bronchial tumor was identified arising from the upper lobe of the left lung, with metastases in mediastinal lymph nodes, epicardium, myocardium, liver, pancreas, adrenal glands, kidneys, and skin. Histologically, metastases of the tumor were also found in the thyroid and parathyroid glands and the heart. The tumor was extremely pleomorphic and primitive but clearly epithelial in pattern, with large basophilic nuclei. The cells had scanty cytoplasm and grew in solid sheets or cords, with many mitoses.

The brain weighed 1,350 gm. and displayed no external alterations. Careful serial slicing of the brain showed no gross abnormalities. The pineal body was normal in size, shape, and consistency (Fig. 1). The pineal tissue was almost completely replaced by metastatic carcinoma, identical in all respects with that seen elsewhere (Figs. 2 and 3). Often only corpora arenacea

Fig. 1 (Case 1).—The brain. The configuration of the pineal body is normal. × 1.3.

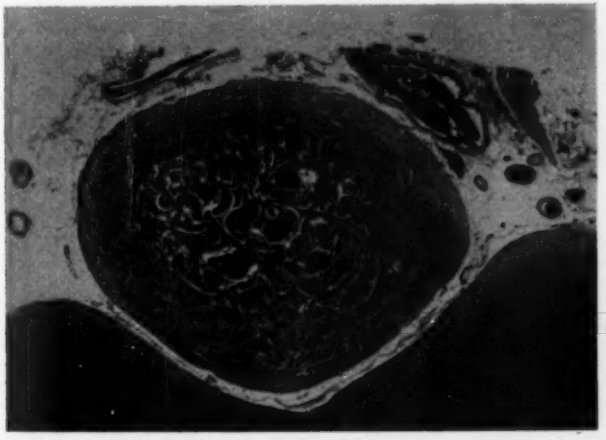

Fig. 2 (Case 1).—The pineal body. The regular outlines are well shown, without involvement of the surrounding meninges or brain substance. Persisting corpora arenacea can be identified within the central portions. Hematoxylin and eosin; × 9.

remained. At some levels a thin rim of pineal body still persisted. There was no invasion of the surrounding structures, and a comprehensive microscopic survey of the brain revealed no other tumor.

Case 2.—A. R., a woman, 62, had had a tumor of the left breast for five years, with involvement of the axilla and ulceration of the skin. Roentgenograms of the chest four years previously

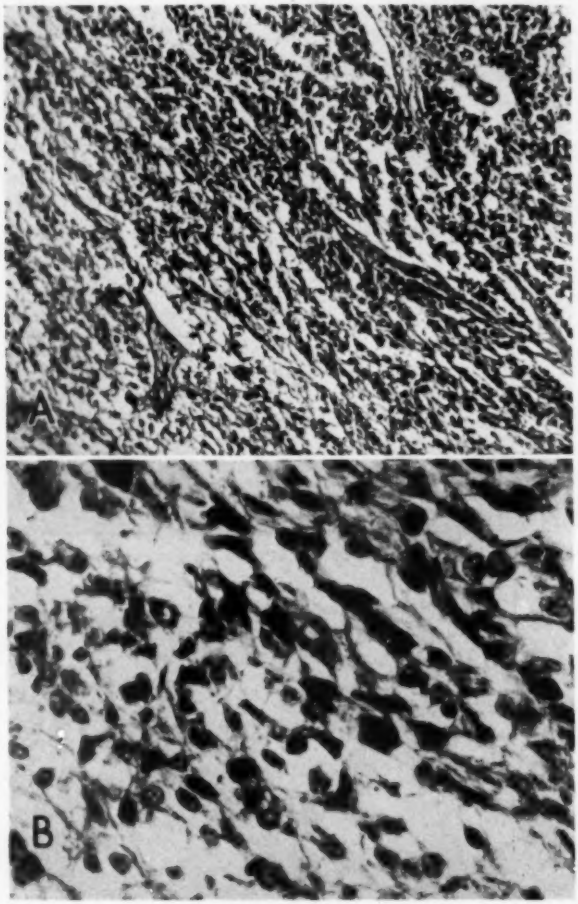


Fig. 3 (Case 1).—*A*, section through the margin of the metastatic carcinoma. Most of the pineal tissue has been destroyed, only a thin rim of pineal tissue being preserved in the left lower corner. Hematoxylin and eosin; × 100.

*B*, cellular detail of *A*. The tumor is pleomorphic and poorly differentiated, with large, hyperchromatic nuclei. Pineal tissue is seen in the left lower corner. Hematoxylin and eosin; × 460.

showed increased pulmonary markings and enlargement of the hilar lymph nodes. The patient had been institutionalized for schizophrenia for 30 years, but evaluations of the mental state and neurologic examination failed to reveal any alterations that would suggest that the brain was involved by tumor.

Fig. 4 (Case 2).—The brain. There is pineal enlargement with compression of nearby structures. × 1.3.

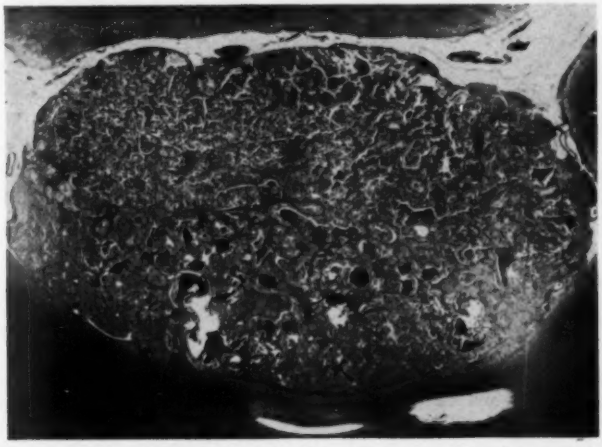

Fig. 5 (Case 2).—The pineal body. There is marked compression of the aqueduct, without brain or meningeal invasion. Corpora arenacea are present. Hematoxylin and eosin; × 6.

Autopsy showed emaciation and a large, hard, nodular mass in the left breast, which was removed for examination. The thorax and the abdomen were not examined.

The brain weighed 1,162 gm. The leptomeninges were slightly thickened, and there was a suggestion of mild cortical atrophy. Careful gross examination otherwise revealed no abnormalities. The pineal body was enlarged to 1.7 by 1.5 by 1.2 cm. and was occupied by a solid, granular,

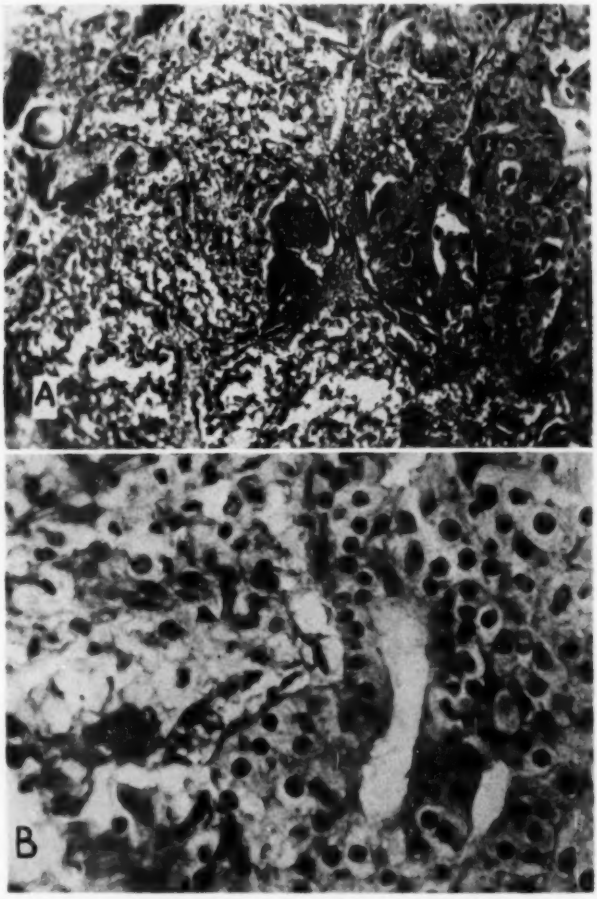


Fig. 6 (Case 2).—*A*, margin of the metastatic carcinoma. There is clear histologic distinction between tumor and normal pineal tissue. Hematoxylin and eosin; × 100.

*B*, cellular detail of *A*. The tumor tissue shows several well-formed glands with persisting pineal tissue on the left. Hematoxylin and eosin; × 460.

grayish tumor (Fig. 4). It compressed the posterior commissure and the habenular nuclei; the superior colliculus and the adjacent pulvinar were slightly displaced. The aqueduct was narrowed but patent. Microscopic examination of the breast tumor showed a carcinoma composed of large eosinophilic cells with oval, slightly vesicular nuclei. Moderate pleomorphism,

a well-developed scirrhous reaction, and occasional glandular areas were noted. The pineal body showed an identical tumor pattern with a thin peripheral rim of persisting normal pineal tissue (Figs. 5 and 6). Although the surrounding structures were compressed, no actual invasion had occurred. Examination of multiple areas of the brain demonstrated no metastases.

CASE 3.—B. E., a woman, 46, had undergone left radical mastectomy for carcinoma nine years previously. Six years later she noted abdominal distention and edema of the legs. Physical examination showed a mass in the pelvis, and roentgenograms revealed extensive metastases in bone and in the lungs. The course was progressively downhill, with hepatic enlargement, emaciation, and recurrent ascites. At no time were there any neurologic signs or symptoms. Autopsy revealed studding of the entire peritoneal cavity with white tumor nodules, which extrinsically involved the intestinal tract. Multiple metastases were present in the liver, heart, spleen, adrenals, ovaries, bone marrow and the anterior lobe of the hypophysis. Microscopic

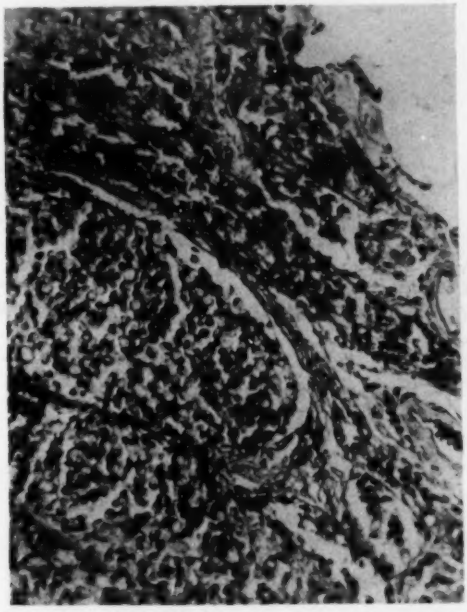

Fig. 7 (Case 3).—The pineal body. A well-formed signet cell type of pattern distinguishes the metastatic carcinoma from pineal tissue, which forms an intact capsule over the carcinoma. Hematoxylin and eosin; × 100.

examination established that the tumor was a mucus-producing carcinoma showing moderate pleomorphism. The cells grew in solid sheets with a scirrhous stromal reaction.

The brain weighed 1,300 gm. and showed no abnormalities on external examination or after serial slicing. The pineal body was grossly normal in size, shape, and position. Numerous sections through the brain showed no metastatic carcinoma except in the pineal tissue, which was partially replaced by a tumor identical with that seen elsewhere (Fig. 7).

CASE 4.—H. S., a white man, 52, had a history of sudden onset of weakness of the left side of the body of three months' duration. Two months later he began to have severe headaches. Physical examination revealed generalized lymphadenopathy, dulness in the right upper lung field, and hypertension (blood pressure, 215/140). There were cardiac enlargement and clubbing of the fingers. Neurologic examination disclosed confusion, poor coordination, and hypesthesia

Fig. 8 (Case 4).—The pineal body. The great bulk of pineal tissue is uninvolved. Two small metastatic deposits of adenocarcinoma are seen within its substance. Hematoxylin and eosin; × 100.

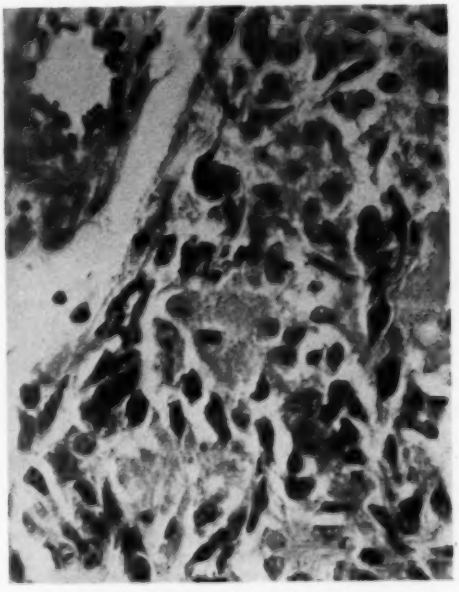

Fig. 9 (Case 5).—Pineal body. Most of the metastatic carcinoma on the right could be encompassed within a high-power field. The tumor cells are spindle-shaped, with large, hyperchromatic nuclei; at least three mitotic figures can be seen. Hematoxylin and eosin; × 460.

of the left extremities, paresis of the left side of the face, and deviation of the tongue to the right. The spinal fluid contained 45 lymphocytes and 116 mg. of protein per 100 cc. of fluid. Roentgenograms showed replacement of the right anterior upper lung field by a lobular mass and a shift of the pineal body to the left. Biopsy of a supraclavicular lymph node showed metastatic adenocarcinoma. The patient's condition deteriorated, and he died three months later.

Autopsy revealed a tumor mass, 6 cm. in diameter, replacing the upper lobe of the right lung, with metastases in the mediastinal lymph nodes. Small metastatic nodules were noted in lymph nodes, the liver and one adrenal. Microscopic examination established that the tumor was a pleomorphic adenocarcinoma composed of large primitive cells with many mitoses.

Gross examination of the brain showed a 3-cm. metastatic nodule in the right cerebrum and a 2-cm. nodule in the right cerebellum. The meninges, spinal cord, and pineal body were grossly normal. The brain masses had a pattern similar to the primary lesion, and the meninges were also invaded. The pineal body contained two small, discrete metastases (Fig. 8).

CASE 5.—P. J., a white man, 61, had had a history of bronchiectasis for 15 years. Two weeks previous to hospitalization he began to have nausea, malaise, and dizziness, associated with brief episodes of unconsciousness and an increasingly productive cough. Physical examination showed pulmonary emphysema, with pulmonary rales. Clonic convulsions affecting the left arm and leg were noted. Roentgenograms of the chest demonstrated emphysema and pulmonary fibrosis.

Autopsy showed an infiltrative tumor involving the left hilum and mediastinal lymph nodes. Invasion of the pancreas, liver, spleen, adrenals and the anterior lobe of the hypophysis was present. Microscopic examination demonstrated an undifferentiated epithelial tumor composed of pleomorphic cells with large nuclei and many mitoses.

The brain had normal external contours, but on routine slicing a cystic lesion, 2.5 cm. in diameter, was found within the right frontal lobe. The pineal body was of normal size and appearance. It showed a small, single, metastatic deposit within its substance (Fig. 9).

## COMMENT

The purported rarity of metastasis to the pineal body is not borne out by our findings. Five instances of metastatic involvement of this structure were found among 130 brains from patients who died of disseminated neoplastic disease.

Of these five cases, three concerned men and two women, although our material was greatly weighted with male patients. Two types of neoplasm were represented: The three men had bronchogenic carcinoma and the two women died of carcinoma of the breast. All five had multiple visceral metastases, and two had clinical and pathologic evidence of intracranial neoplastic involvement.

The striking result of the study was that three of the metastases represented apparently isolated transference of tumor cells to the pineal body without other involvement of the brain or the meninges. These three cases showed massive neoplastic replacement of the pineal body. In contrast, the two cases in which the brain and meninges were involved showed only microscopic seeding of the pineal body by tumor. In none of the patients was there clinical evidence suggestive of involvement of the pineal body. This would confirm the observation that pineal lesions produce symptoms only by compression of neighboring structures.

Thus, isolated pineal mestastases were found in 3% of 98 brains otherwise free of tumor, and in 6% of brains which had multiple sites of intracranial metastases. We are forced to conclude that respecting metastatic spread of neoplasm the pineal body has a feature that distinguishes it from a fortuitous site of tumor cells disseminated to the central nervous system. A plausible mode of localization of pineal metastasis is suggested by consideration of the blood supply of the pineal body. Arteries to this structure are derived from the posterior choroidal branches of the

two posterior cereebral arteries, which have the vertebral arteries as their source.[3] Since the central nervous system in the area of the pineal body does not possess a lymphatic vessel system, the only alternative course of tumor emboli might be through the vertebral veins, as described by Batson.[4]

SUMMARY

Five cases of metastasis to the pineal body were found among 130 brains from patients with widespread neoplastic disease. In three there were no other intracerebral metastases.

The incidence of pineal metastases in patients with widely disseminated neoplastic disease was 4%.

3. Gladstone, R. J., and Wakeley, C. P. G.: The Pineal Organ, Baltimore, Williams & Wilkins Company, 1940, p. 528.

4. Batson, O. V.: The Function of the Vertebral Veins and Their Role in the Spread of Metastases, Ann. Surg. **112**:138-149, 1940.

# A STUDY OF TISSUE IMPLANTATIONS ON EMBRYONATED-EGG MEMBRANES

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THE CHORIOALLANTOIS of the chick embryo has been utilized for grafting tumor tissues and for culturing viruses, rickettsiae, and protozoans. Murphy [1] demonstrated the possibility of grafting certain cancers of the rat and the mouse on the membranes of embryonated eggs. Goodpasture, Douglas, and Anderson [2] grafted human skin on this membrane. Goodpasture and Anderson [3] studied fowlpox immunity, virus infections of human fetal membranes, and herpes zoster infections on this culture medium. Cox [4] utilized it for the culture of rickettsiae. Blank, Coriell, and McNair Scott [5] grafted human skin on the chorioallantois of the chick for herpes and vaccinia virus cultivation.

The above research very well proved the value and practicability of the chorioallantois of the chick as a good medium for tissue cultivation, and seemed to us suited for the study of various tissue implantations, namely, those of chick tissues and various human tissues. The object of these investigations was to determine, first, what happens to the grafted tissues and, second, what are the reactions of the chorioallantois.

The investigations were arranged in two groups: (1) human tissue implantations; (2) chick embryo implantations.

## MATERIALS AND METHODS

The human tissues were obtained at surgical operation and immediately immersed in a saline solution to which was added 50,000 units of penicillin per cubic centimeter. The tissue was kept at body temperature and was implanted within 10 minutes to one hour. Chick tissue was obtained from chick embryos varying in age from 8 to 20 days of incubation. These tissues were

1. Murphy, J. B.: Transplantability of Malignant Tumors to the Embryo of a Foreign Species, J. A. M. A. **59**:874 (Sept. 14) 1912.

2. Goodpasture, E. W.; Douglas, B., and Anderson, K.: A Study of Human Skin Grafted upon the Chorioallantois of Chick Embryos, J. Exper. Med. **68**:891, 1938.

3. Goodpasture, E. W., and Anderson, K.: Immunity to Fowlpox Studied by Means of Skin Grafts on Chorioallantois of Chick Embryos, Arch. Path. **30**:212 (July) 1940; Virus Infection of Human Fetal Membranes Grafted on the Chorioallantois of Chick Embryos, Am. J. Path. **18**:563, 1942; Infection of Human Skin, Grafted on the Chorioallantois of Chick Embryos, with the Virus of Herpes Zoster, ibid. **20**:447, 1944.

4. Cox, H. R.: The Cultivation of Rickettsiae in the Tissues of Developing Chick Embryos, Pub. Health Rep. **54**:2171, 1939; Science **94**:399, 1941.

5. Blank, H.; Coriell, L. L., and McNair Scott, T. F.: Human Skin Grafted upon the Chorioallantois of the Chick Embryo for Virus Cultivation, Proc. Soc. Exper. Biol. & Med. **69**:341, 1948.

implanted immediately without any treatment. Embryonated eggs of 9 to 12 days' incubation were used as recipients of the implants. After tincture of iodine had been applied to the shell, the air sack was ruptured, and a square window about 6 by 12 mm. was cut into the shell with the aid of a dental disk. The shell membrane was reflected with care to protect the underlying egg membrane. The implants, which varied from 1 to 4 cu. mm. in size, were carefully placed on the chorioallantoic vessels. The window was sealed with scotch® cellulose tape, and celloidin (pyroxylin) was applied about the margins of the tape and the opening of the air sack. Incubation was then continued for various periods. When the eggs were opened, their viability was determined; the implant was carefully examined as to the manner in which it was related to the membrane and surrounding vessels. The tissue was fixed *in situ* with 10% formalin and then excised with a reasonable margin of attached membrane, sectioned, and stained with hematoxylin-eosin.

### MACROSCOPICAL APPEARANCE OF THE IMPLANT

If the eggs survived, the implants appeared macroscopically more or less enveloped by the chick membrane. In most instances, large vessels of the membrane were converging on the implant. Some of these vessels appeared engorged, and in many instances there appeared to be a continuous "halo" of extravasated blood marginating the implant. After fixation and excision the implant could frequently be seen protruding above and below the membrane, and in many instances there was an obvious increase in the size of the implant. Too, some of the implants showed one to several vesiculations. The vesicles varied in size and protruded into the allantoic sac from the deep surface of the implant.

### HUMAN TISSUE IMPLANTS

1. *Tonsillar Tissue.*—(*a*) A tonsillar implant was incubated for 7½ days. The implant was adherent to the allantoic membrane along the entire base. Large allantoic vessels were converging on the implant, penetrating into it at its base. A large edematous border surrounding two-thirds of the implant's circumference was obvious.

Low-power microscopic examination revealed the implanted tonsillar tissue to be completely engulfed by the membrane (Fig. 1*A*). The implant elicited a surrounding tissue reaction, evidenced by increase in the cellular elements about the implant. In some areas this reaction was rather prominent, suggesting an accumulation of macrophages which formed a dense line of rather large cells which in turn surrounded a zone of paler elements that suggested liquefaction. The central portion of the implant was uniformly dense and appeared to be very cellular. The sections revealed, too, that the epithelium of the allantoic membrane was intact and that the implant and the surrounding reaction were totally interstitial. Other implants showed essentially the same structure under low-power magnification.

High-power magnification of a part of the same area (Fig. 1*B*) revealed a margin of the implanted tissue and the surrounding allantoic tissue reaction. The bordering tissue was very cellular and very vascular, as evidenced by numerous small vessels filled with chick red blood cells. The zone of dark cells surrounding the implant consisted of a thick layer of cells with poorly defined cellular membrane but with large hyperchromatically stained nuclei, which varied in size and appeared to be pseudostratified. These cells marginated the clear zone, which was acellular and of fibrillary appearance. The underlying implant was granular, suggesting necrosis, with only ghost remains of the cellular structure of lymphoid tissue.

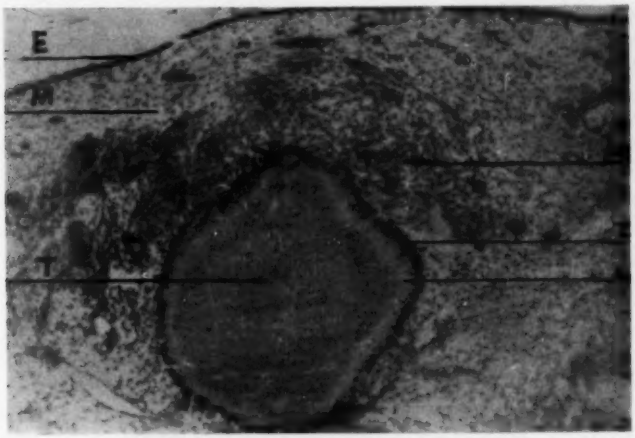


Fig. 1A.—Tonsillar implant, low-power photomicrograph. *E* indicates epithelium and *M* stroma of allanotic membrane; *T*, tonsillar implant; *R*, tissue reaction in chick membrane; *Z*, zone of macrophages; *L*, zone of liquefaction.

Fig. 1B.—Tonsillar implant, high-power photomicrograph. *T* indicates tonsillar parenchyma; *L*, zone of liquefaction; *Z*, zone of macrophages; *V*, chick vessel; *M*, stroma of allantoic membrane.

Oil immersion (Fig. 2) of the same section showed small capillary loops marginating the dark zone of cells. The dark cells had granular cytoplasm with a poorly defined cell membrane. In some of the cells the nucleus was vesicular and contained a nucleolus, while in other cells the nucleus was of poorly defined nuclear material, which in some areas was highly suggestive of giant cell structure. Granular material, the granules of which varied considerably in size, was noted in the cytoplasm of all these cells. This material was most evident in the cells which appeared along the border of the light zone. This zone, which was clear and called by us the "zone of liquefaction," was not entirely composed of amorphous material, but in some areas

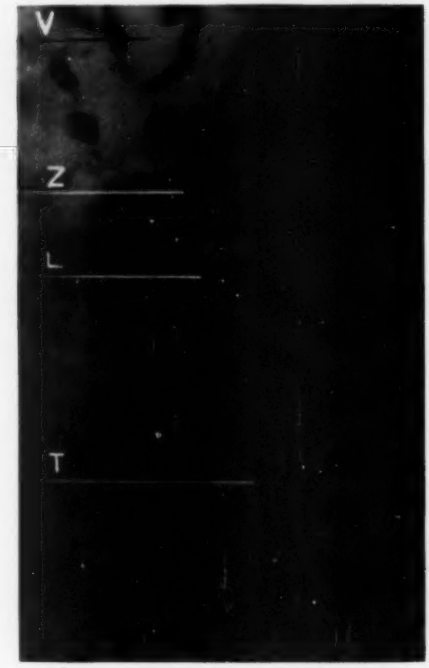


Fig. 2.—"Zone of liquefaction"; oil immersion. *T* indicates tonsillar parenchyma; *L*, zone of liquefaction; *Z*, line of macrophages; *V*, chick vessel.

appeared to be granular and vacuolated. This granular material was sparse and not nearly as dense as that in the cytoplasm of the macrophages or that in the internal structure of the implant, but was none the less of a similar granular appearance. The portion of the implant marginating the zone of liquefaction showed large granules suggesting nuclear debris and granular material. Two interpretations were suggested on examination of this reaction: first, that the surrounding large cells were possibly secreting a substance which dissolved the implant and thence allowed or aided phagocytosis; second, that this light zone represented a zone of the implant from which the nuclear debris has been removed by the macrophages.

Another field of the same tonsillar implant which was covered by squamous epithelium showed chick membrane completely engulfing the implant. The membrane was very vascular with striking variation in the sizes of the vessels. This very vascular membrane rested on the squamous epithelium of the implanted tonsil. The membrane elicited no reaction in this area. The squamous epithelium of the implant appeared to be well preserved and in turn covered the typical tonsillar tissue, which showed the usual necrotic structure.

High-power magnification of this junctional zone between the squamous epithelium and the membrane epithelium revealed the membrane to be composed of loose reticular cells which were poorly vascularized and were in pronounced contrast to the above tissue reaction seen when tonsillar tissue was marginated by membrane. The squamous epithelium was composed of relatively well preserved squamous cells which had well-defined nuclei. The lymphoid tissue beneath this epithelium presented the usual necrotic appearance, including nuclear debris and poorly stained other elements. The interpretation of this reaction strongly suggests that the squamous epithelium of the implanted tonsillar tissue prevented or aborted the reaction elicited when tonsillar lymphoid tissue immediately adjoined the membrane.

(*b*) Tonsillar tissue was kept in saline solution as a control for *a* and was treated with penicillin as was *a*. This tissue, after being treated as above, was stored in an incubator at 37 C. for seven days.

Stained sections of this tissue showed deeper eosinophilic staining qualities than the tissue which had been transplanted. An occasional nucleus, however, stained basophilically like those of the transplant. Probably there was a marked increase of nuclear debris, too.

2. *Nasal Polyps.*—Pieces of nasal polyps, after being removed from the patient, were placed in saline solution for three hours and then were implanted into embryonated eggs. The eggs were alive after three days' incubation, and the tissue appeared adherent at the base to the chick membranes.

Low-power magnification (Fig. 3) showed the allantoic membrane to be firmly affixed to the implant. The allantoic tissue adjacent to the implanted polyp was very vascular, and these small vessels appeared to be streaming toward the polyp. In some areas these vessels appeared to be invading the polyp parenchyma. Some of the larger vessels which were engorged with chick red blood cells had some channels adjacent to their adventitia, and no cellular elements were noted in these channels; however, it was thought that the channels probably were lymphatics.

Some islets of lymphoid tissue were noted in the polyp, eliciting a tissue reaction similar to that observed in the tonsillar tissue implants. Large phagocytic cells were noted surrounding the lymphoid tissue, marginated internally by a zone of so-called liquefaction or phagocytosed cells.

3. *Human Cornea.*—A large piece of a cornea of a patient who had sustained a penetrating injury of the eye 10 days previous to surgical operation, with subsequent development of iridocyclitis and cataract, was implanted. The egg was alive after six days' incubation. The implanted cornea was adherent and almost completely engulfed; thick vessels radiated concentrically to the implant and partially traversed it. The piece of cornea appeared slightly larger than when it was implanted. After fixation and excision the implant seemed adherent to the mem-

brane, the two appearing as one unit, and the membrane about the implant was much thicker than the surrounding membrane.

Low-power magnification (Fig. 4, upper part) showed the implanted cornea completely surrounded by the supporting stroma of the chick membrane. Chick membrane capillaries entered the implanted cornea in several areas. These vessels exclusively invaded the cornea parallel to and between the collagen bundles of the cornea. At no point did these vessels enter or invade in a perpendicular plane (Fig. 4, middle part). At the site where the metaplastic squamous epithelium of the chick membrane adjoined the corneal squamous epithelium (Fig. 4, lower part) there

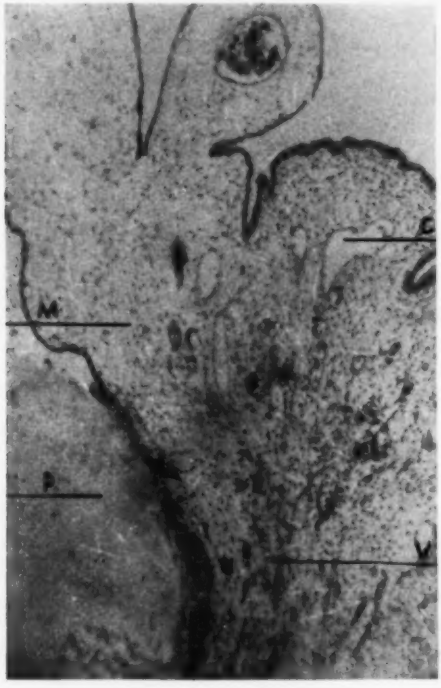


Fig. 3.—Nasal polyp implant. *P* indicates polyp; *M*, stroma of allantoic membrane; *C*, lymphatic channels; *V*, chick vessels streaming toward polyp.

appeared to be no definite line of demarcation or separation of these two epithelia; in fact, some views showed that the epithelium was continuous. Under high-power magnification and oil-immersion (Fig. 5) sections revealed anatomicohistological continuity between these two epithelia.

Sections showing the capillaries entering or invading the implanted cornea showed that these capillaries lay between the collagen bundles and contained nucleated chick red blood cells. In numerous areas nucleated red blood cells appeared without obvious allantoic capillary walls. According to Goodpasture, this implies or indicates that the implanted tissue is alive; however, in view of findings on

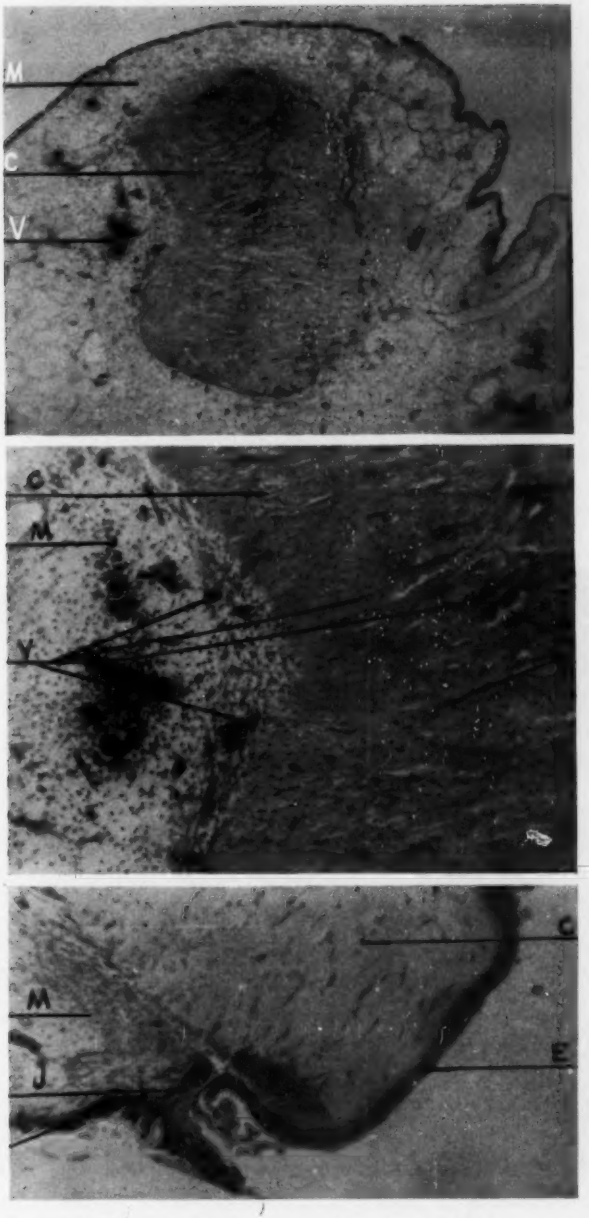


Fig. 4.—Corneal implants. In the low-power photomicrograph (upper section of figure) *C* indicates corneal implant; *M*, allantoic membrane; *V*, chick vessels.

In the high-power photomicrograph (middle section) *C* indicates cornea; *M*, allantoic membrane; *V*, chick vessels near corneal implant and between collagen bundles of cornea; *V*, corneal vessels containing chick blood cells.

In the low-power photomicrograph (lowest section) *C* indicates cornea; *E*, corneal epithelium; *M*, allantoic membrane; $E_1$, allantoic epithelium; *J*, area of continuity of epithelia.

Fig. 5.—Lower section of Figure 4; oil immersion. *C* indicates corneal epithelium; *A*, allantoic membrane epithelium; *M*, continuity of both epithelia.

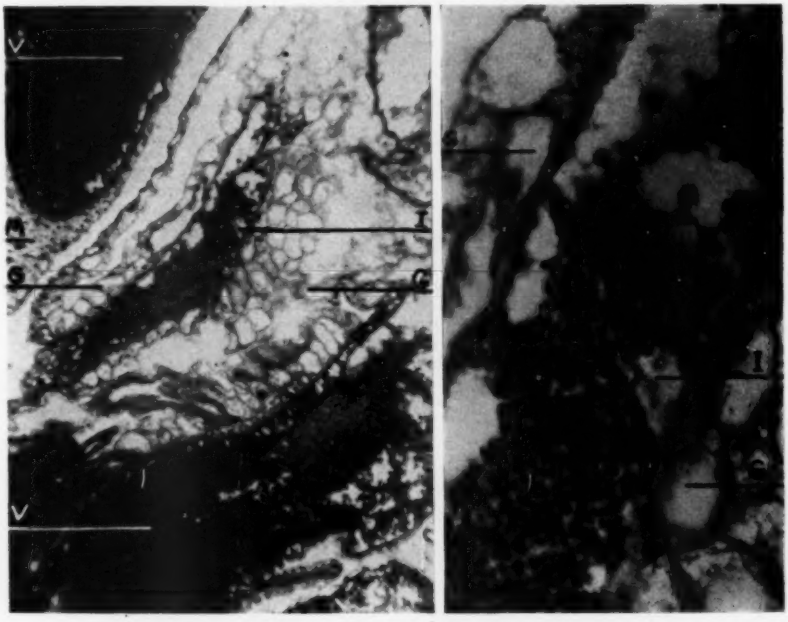


Fig. 6.—Sterile absorbable, water-insoluble gelatin-base sponge implants. In the low-power photomicrograph (left) *G* indicates gelatin sponge (gelfoam®); *M*, allantoic membrane; *V*, engorged vessels of allantoic membrane; *I*, chick membrane vessel invading implant.

In the high-power photomicrograph (right) *G* indicates gelatin sponge; *I*, allantoic membrane vessel invading implant.

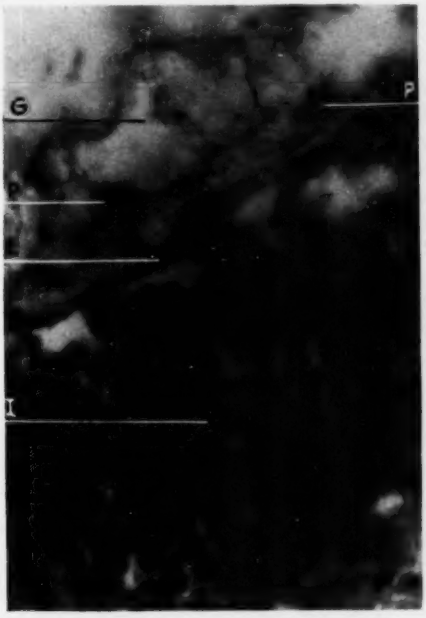


Fig. 7.—Blood vessel invading gelatin sponge implant; oil immersion. *G* indicates gelatin sponge; *I*, vessel lumen filled with chick red blood cells; *E*, endothelium of vessel; *P*, macrophages migrating out into gelatin sponge.

Fig. 8.—Gelatin sponge implant; oil immersion. *C* indicates invading chick membrane capillaries without blood cells.

implanted chick tissues to be discussed later, we differ in opinion in this instance and feel that this possibly represents a phase of organizing foreign body reaction.

4. *Absorbable Gelatin Sponge Immersed in Human Tonsil Extract.*—Small pieces of absorbable gelatin sponge [6] were implanted on the allantoic membrane of six-day-embryonated eggs and allowed to remain for two days. The implant appeared to be engulfed by the membrane, and the vessels appeared to be on the surface of the implant.

Low-power magnification of the implanted sponge (Fig. 6) revealed that the allantoic membrane had completely engulfed it and that the reaction of the vessels was similar to that seen in other implanted tissues, consisting mainly of a marked increase in the vascularization and a marked engorgement of the vessels, with possible thrombosis or "laking" of the larger ones. The implant was flanked by two large vessels, and a blood vessel penetrated the center of the implant. High-power magnification (Fig. 6) showed the invading vessel penetrating the implant.

Oil immersion of this area (Fig. 7) showed the lumen of the vessel described above and its relation to the implant. The endothelium of this vessel was formed by flattened cells with flattened nuclei. The lumen of the vessel was filled with numerous nucleated chick red blood cells. Some large cells, which highly simulated macrophages, with round dark nuclei and large eosin-stained cytoplasm were noted out in the sponge surrounding the vessel and in the neighborhood of the adventitia of the larger vessel. These cells, it is most likely, had originated in the vessel and had migrated out of the wall into the sponge and were in the process of phagocytosing this implanted material. These cells were morphologically very similar to the macrophages seen in the implanted tonsillar tissue in the process of dissolution of the implanted tonsil.

In some zones (Fig. 8) capillary loops were found penetrating the sponge. These capillary loops in some instances did not contain blood cells and apparently represented the earliest stages of invasion and organization.

### CHICK EMBRYO IMPLANTS

1. *Lung.*—(*a*) Lung tissue from a 9-day-old chick embryo was implanted in embryonated eggs 9 days old. These eggs were incubated for five days. The eggs were alive, and the pieces of tissue seemed larger than when transplanted. The implants appeared to be completely covered by membrane, and the vessels converged on the implant from all directions. After fixation the implants appeared engulfed by the allantoic membrane and numerous transparent "blisters" seemed to hang from the implant into the chorioallantoic sac.

Under low-power magnification (Fig. 9) sections revealed the implant to be completely covered or engulfed by the allantoic membrane. There appeared to be a clear zone between the membrane and the implanted lung tissue (artefact?). The lung tissue consisted of numerous tubular structures, bronchioles, which varied considerably in size and all of which were supported by a loose areolar connective tissue stroma.

Under high-power magnification sections showed these tubular structures to be lined with epithelium of a transitional type. This epithelium showed numerous

6. The sterile, absorbable, water-insoluble gelatin-base sponge used was gelfoam,® made by the Upjohn Company, Kalamazoo, Mich.

mitotic figures. The supporting stroma was loose and vascularized. The mitotic activity indicated that the implanted tissue was living and growing when excised.

Under oil immersion the section shown in Fig. 9 disclosed a mitotic figure in the anaphase stage. Other sections showed numerous mitotic figures in the supporting stroma, again indicating that the supporting stroma and the epithelium were living and growing.

(*b*) From a chick embryo 17 days old a piece of lung was taken and implanted in embryonated eggs 9½ days old and incubated six days. The eggs were alive, the implants were completely engulfed, and after fixation they hung down like a

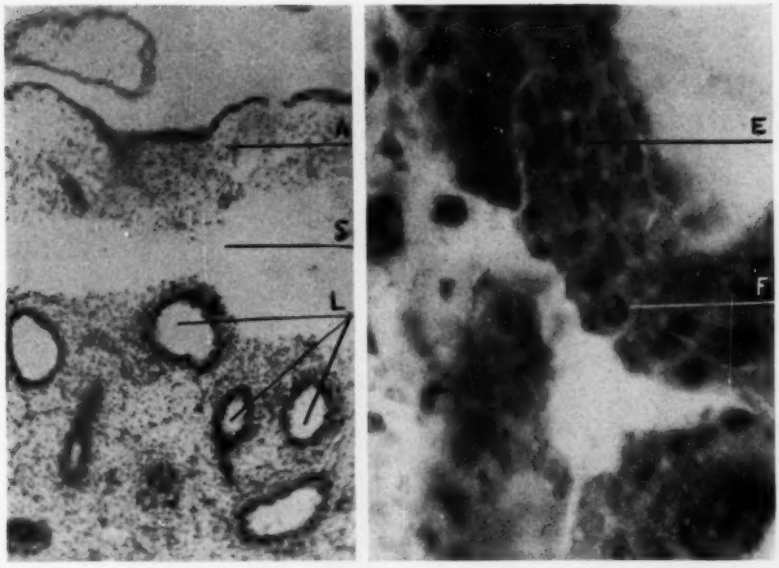


Fig. 9.—Chick lung implants. In the low-power photomicrograph (left) *A* indicates allantoic membrane; *S*, clear zone between allantoic membrane and implant; *L*, bronchioles. In the oil-immersion section (right) *E* indicates bronchial epithelium and *F*, a mitotic figure.

bell from the membrane. Since these implants were 17 days old at the time of implantation and were incubated for six days, these tissues were actually 23 days old.

Under low-power magnification (Fig. 10, left) sections revealed the implanted tissue to be completely engulfed by the allantoic membrane. The implant was alive and showed cartilage, bronchioles with primary alveoli, and supporting stroma.

High-power magnification revealed the epithelium of the bronchi to be composed of well differentiated alive-appearing cells. The cartilage was also well developed cellularly, and mitotic activity was noted in the chondroblasts. Mitotic figures were also seen in the bronchial epithelium. Oil immersion showed mitotic activity, also.

(*c*) Same as *b*, different piece of lung. Under low-power magnification (Fig. 10, right) sections showed many bronchioles cut longitudinally, with numerous

spaces projecting into the bronchiolar walls. These spaces were interpreted to be alveoli or alveolar sacs.

Under high-power all the alveolar sacs were seen to be lined with epithelium similar to that lining the terminal bronchioles but much flatter. The terminal bronchioles had smooth muscle cells in their walls. This smooth muscle was not noted in the walls of the alveoli. It appeared that the alveoli were in the process of formation. Mitotic figures were common in the alveolar epithelium under oil immersion.

Though the implants were from the same chick, they showed better differentiation of the alveolar sacs than *b*. (This could be due to the fact that *b* was a cross

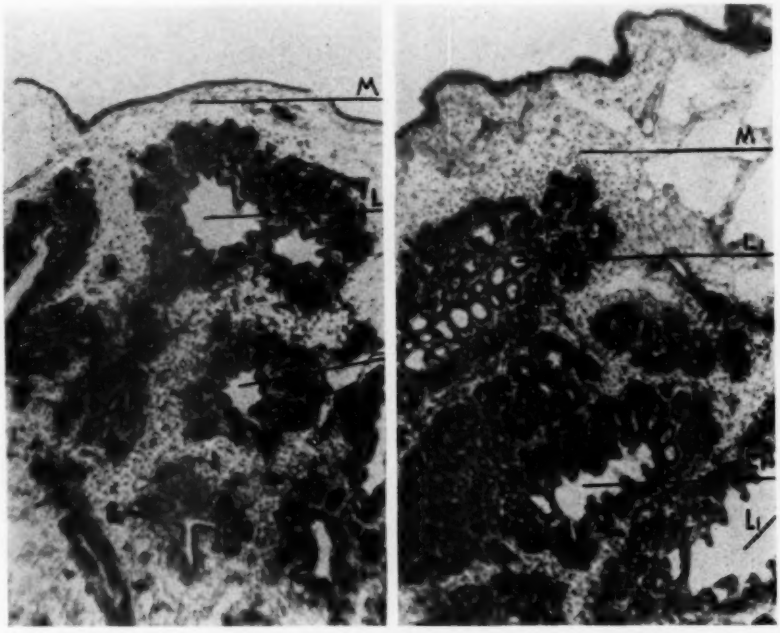


Fig. 10.—Low-power photomicrographs of chick lung implants. *M* indicates allantoic membrane; *L*, bronchioles; $L_1$, bronchioles with alveolar sacs.

section and *c* showed the bronchioles cut in a longitudinal plane.) It appears, too, from these sections that the lung was more developed than it would have been at 17 days and appeared to be ready for expansion seemingly at about 22 days of age, or similar to what one would expect to find at the time of hatching. This, it is thought, indicates that this lung tissue was growing at about the normal rate at which it would have grown in its normal habitat.

2. *Gut.*—(*a*) Gut from a 17-day-old chick embryo was implanted on the membrane of an alive embryonated egg 9½ days old. The implants were allowed to grow for six days. The eggs were alive. The implants appeared to be different from other implants in this series, being greenish.

Under low-power magnification (Fig. 11) sections showed the gut to be completely engulfed by the membrane. The lumen of the gut was patent; the mucosa formed numerous mucosal folds. The tunica propria of these folds or villi was very cellular, and the muscular coat appeared to be well differentiated. Three small tubular structures (biliary ducts) lined with epithelium were noted in the zone of what appeared to be the subserosa.

Under high power, sections showed the epithelium to be of a tall columnar type resting on a basement membrane. The tunica propria showed many rounded, darkly stained nuclei which simulated lymphocytes. These cells were scattered throughout the tunica propria, with no apparent germinal follicle formation. In other sections numerous mitotic figures were noted in the epithelium and in the smooth muscle.

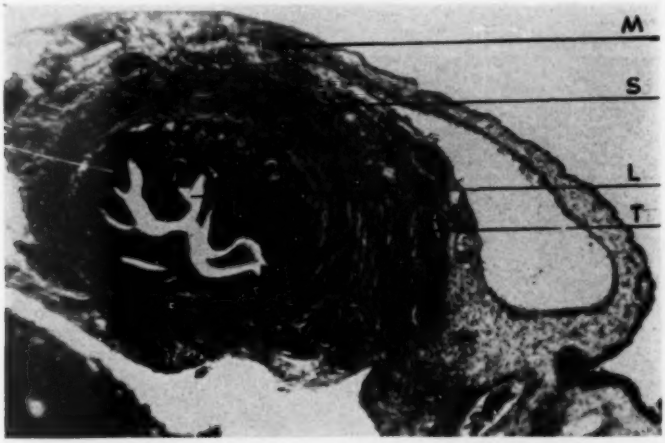


Fig. 11.—Chick gut implant; low-power photomicrograph of gut from a 17-day embryo. *M* indicates allantoic membrane; *S*, muscular coat; *F*, tunica propria of villi; *L*, lumen; *T*, tubular ducts.

(*b*) Gut from a 17-day-old chick embryo was implanted on the membrane of 9-day-old embryonated eggs and was incubated for six days. The eggs were alive, and the implants grossly appeared as convolutions of tubular structure.

Under low-power magnification sections of the implanted gut showed complete encapsulation by the membranes, and the tissue appeared to be alive and showing no evidence of necrosis. Mitotic figures were numerous throughout the whole wall of the gut. Cross sections of the gut showed all layers to be well preserved, epithelium, tunica propria, muscularis mucosa, submucosa, lymphocytes, muscularis, subserosa, and serosa. Under high-power magnification sections showed very clear and distinct autonomic ganglia (Fig. 12) (Auerbach's and Meissner's plexuses) in their usual position. These plexuses had well developed ganglion cells.

3. *Cornea.*—From a chick embryo 10 days old one entire cornea was implanted along with a piece of conjunctiva and sclera. After six days of incubation the implanted tissue appeared entirely engulfed by chick membrane. The implant

seemed to consist of four parts, which were attached to each other in a "mosaic" pattern. While the implant appeared fairly pale, streaks of pigment were radiating from it along the vessels.

Sections (Fig. 13, upper part) revealed that the allantoic membrane epithelium marginated and became continuous with the corneal epithelium. The external corneal epithelium was intact; the cornea was composed of stellate cells embedded in a fine fibrillar connective tissue mesh. Deep to it lay a large round space filled with many cells having dark nuclei and eosinophilic cytoplasm (posterior chamber?). The space was marginated by pigmented columnar epithelial cells. The cornea was laminated; fibers were distinctly visible and were vacuolated especially in the periphery. Nuclei could be distinctly seen. A portion of ciliary body was noted; it formed numerous papillary processes lined with pigmented cells; smooth muscle cells and free pigmented cells were also embedded in the main body. Two

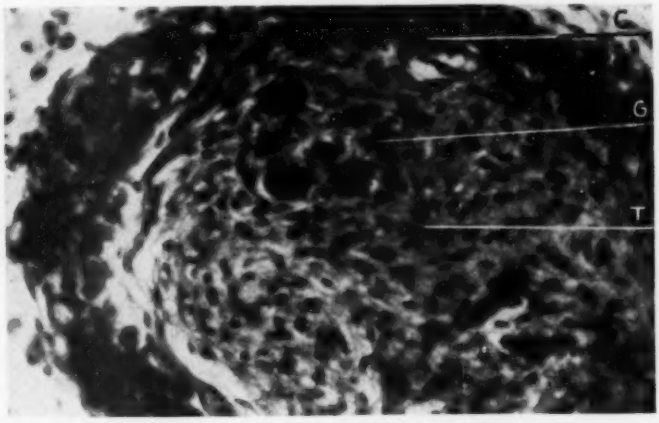


Fig. 12.—High-power photomicrograph of an autonomic ganglion of gut from a 17-day-old chick embryo. *C* indicates ganglion capsule; *G*, ganglion cells; *T*, nerve bundles.

hyaline cartilaginous plates were found in the margin of the implanted tissue (tarsal or orbital cartilage?). Figure 13 (lower part) shows a high-power photomicrograph of the corneal stroma.

The implanted tissue was marginated by granulation tissue, and all vessels within the implanted tissue contained normal-appearing chick red blood cells. The tissue was alive, as revealed by mitotic activity. The chick membrane showed a granulation tissue reaction at the site of junction.

4. *Spleen.*—The spleen of a chick embryo 15 days old was implanted. After five days' incubation the implant was completely engulfed by chick membrane and thick vessels were noted converging on it. The implant appeared larger and thicker at the time of excision than at the time of its implantation.

Microscopic section showed the tissue completely engulfed, eliciting little if any reaction from the surrounding chick membrane. The implanted tissue showed

a margin of alive splenic parenchyma with vaguely recognizable splenic nodules, both white and red pulp. A central zone composed of shrunken, poorly defined cells was noted, simulating what previously was thought to be necrotic implanted tissue.

5. *Liver.*—A piece of liver was implanted from a chick embryo 12 days old. After five days' incubation the implant was completely engulfed by chick membrane,

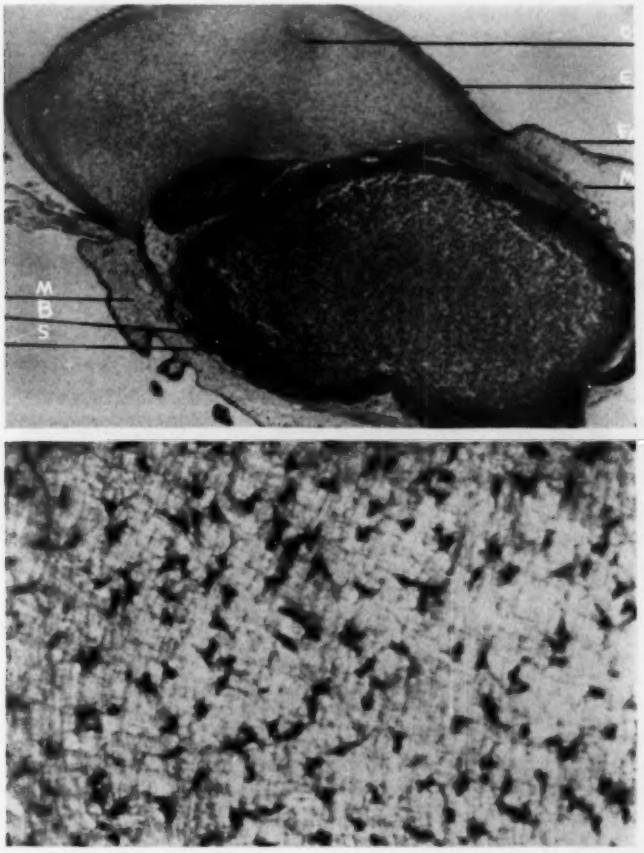


Fig. 13.—Chick cornea implant. In the low-power photomicrograph (above) *C* indicates cornea; *E*, corneal epithelium; $E_1$, allantoic membrane epithelium; *M*, allantoic membrane; *B*, ciliary body; *S*, posterior chamber.

In the high-power photomicrograph (below) the stellate cells and fibrillar connective tissue are shown.

did not project over the surface at all but extended inwardly. Large vessels converged on the implant as noted in other implants.

Microscopic sections (Fig. 14, upper part) revealed an intact membrane completely engulfing the implanted liver. Granulation tissue marginated the deeper

strata of the implant. The implant showed a central early necrosis with only a ghost structure of liver parenchyma remaining. The periphery of the implant, however, showed well-vascularized, rapidly growing, normal-appearing parenchyma, hepatic cords, and vascular channels. Mitotic figures were numerous. Under high-power magnification (Fig. 14, lower part) sections showed well-preserved, alive hepatic cord cells.

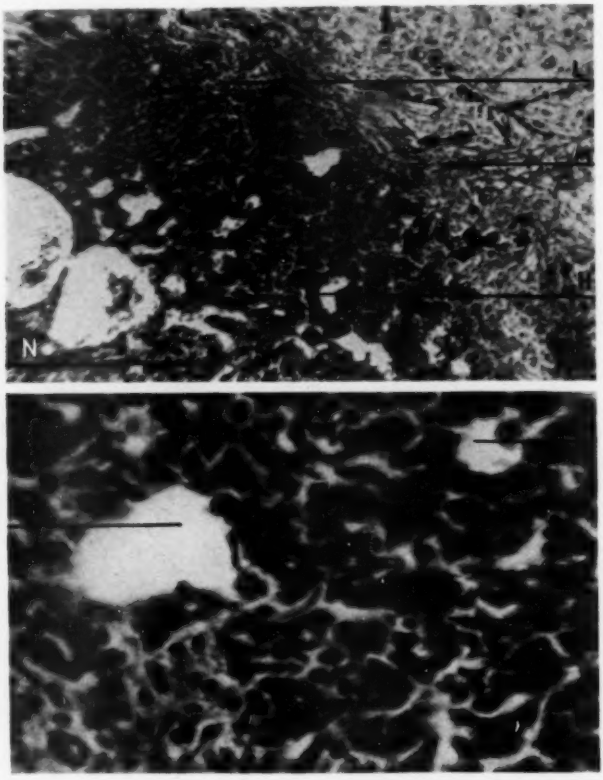


Fig. 14.—Chick liver implant. Low-power photomicrograph above; high-power photomicrograph below. *L* indicates live liver cells in periphery; *M*, allantoic membrane; *H*, vascular channels; *N*, central necrosis of implant.

## COMMENT

The chorioallantoic membrane of embryonated eggs was used in these investigations to transplant different types of tissue of human and chick origin and to study the fate of the tissues as well as the reaction of the membrane. Transplantation was successful in many instances, and the fate of the implant and the reaction in the membrane varied according to the tissue implanted and according to whether or not the implanted tissue remained alive. If the implant survived, it frequently appeared

increased in size. The membrane in general engulfed the implant, and the membrane vessels in the vicinity of the implant appeared engorged and converging on the implanted tissue.

In human implants a varying degree of dissolution was noted, due to lytic and macrophagic processes elicited by the surrounding necrotized portions of the implant; in other areas granulation tissue appeared, with development of fibrosis. The chick capillaries frequently invaded the tissue spaces and blood vessels of the implant, in which chick red blood cells could be found. This phenomenon in our opinion does not necessarily indicate that the implant was alive as concluded by Goodpasture. We feel that mitotic activity, growth, and viability of the cellular components of the implant are more conclusive evidence of viability of the tissue.

The epithelium of the implant in general was more resistant to dissolution, seemed to elicit little or no macrophagic reaction, and in some tissues appeared to become confluent with the allantoic membrane epithelium.

The fate of the interstitial tissue of the implant varied. Tissue containing abundant collagen connective tissue bundles appeared to resist dissolution and organization, whereas lymphoid tissue appeared to lose its normal structure and become homogeneous and, in some areas, necrotic.

The chick membrane reaction in general was one of varying degrees of organization. This granulation tissue reaction was especially notable when the implant was of lymphoid origin, and this reaction consisted of extensive production of macrophages. It could best be studied in its first stages in absorbable gelatin sponge implants where capillary buds invaded the implant preliminary to granulation.

Chick tissue implants remained alive in a high percentage of cases. If the implant lived, the structure was well preserved; mitoses could be found in all elements of the implanted tissue; differentiation of tissues continued, and many of the implants revealed gross evidence of growth.

## CONCLUSIONS

Tissues of various organs of human and chick origin can be implanted on embryonated-egg membrane. The implants can be maintained alive for several days, and chick tissue will even grow and continue to develop.

The chick membrane shows reactions which are identical with human mechanisms. It shows phagocytosis, absorption, organization; all of these functions are associated with the membrane capillaries, which appear to form the most important constituents of the chick membrane. The latter possesses these qualities even in the first days of the chick embryo's life.

# CHOLESTEROL IN PLASMA AND TISSUES SUBSEQUENT TO ITS INTRAVENOUS INJECTION

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CHOLESTEROL is a normal constituent of the cells and fluids of the body, where it exists in the free state and also combined with fatty acids as esters. The amount found is not equally distributed in the different tissues. The brain and adrenal glands have the richest supply, with various amounts in the other tissues.

The influence of diet on the cholesterol content of blood and tissues has probably received more attention than any other factor. However, cholesterol has been fed to animals, and both by chemical analysis of tissues and microscopic examination, deposits of cholesterol have been demonstrated. Rats,[1] guinea pigs,[2] rabbits,[3] dogs,[4] and chickens [5] have been used, with deposits found in liver, kidney, lung, adrenals, and aorta.

Attempts to influence the cholesterol content of blood and tissues by intravenous injections have been reported. Cashin and Moravek [6] injected colloidal cholesterol with lecithin into cats. They found blood cholesterol decreased 20% from normal in some animals and as much as 60% in others. The animals became insensitive to pain, and paralyzed. Respiratory rate increased and heart rate decreased. Chemical analyses of tissues revealed an increase of cholesterol in the lung and some increase in the liver.

Block, Berg, and Rittenberg [7] administered cholesterol containing deuterium, using equal parts of the sterol and lecithin suspended in a phosphate buffer, into the femoral vein of a dog in which an anastomosis between the gallbladder and the pelvis of the kidney had been established. The distribution of the administered

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cholesterol was determined in the various organs. The highest concentration of deuteriocholesterol was found in the lung. The next highest was in the liver.

The relation of cholesterol and pulmonary function was suggested by a group of French workers [8] 30 years ago. They reported blood of the right side of the heart richer in cholesterol than that of the left. It therefore seemed to them that cholesterol was either fixed or destroyed in the lungs. On the other hand, Shillito, Bidwell, and Turner,[9] in 1936, in this country, could find no differences in blood cholesterol in the carotid artery, the venae cavae, and the portal vein.

The purpose of this study was to determine the effect of repeated intravenous cholesterol injections on the cholesterol content of the blood and various tissues of dogs.

## EXPERIMENTAL PROCEDURE

Sixteen dogs were used in this study. With six of the dogs, weekly injections of cholesterol were started after a preliminary control period of two weeks and continued for six months.

The difficulties encountered in preparing a suspension of cholesterol are related to preserving the suspension in a properly divided state for a considerable length of time. As a result, various suspensions that are injected may show alterations in particle size and clumping of particles

TABLE 1.—*Amount of Cholesterol Injected Weekly at Rate of 50 Mg. per Kilogram of Body Weight*

| Dog | Weight, Kg. | Cholesterol Injected, Mg. |
|---|---|---|
| 1 | 9.4 | 470 |
| 2 | 8.8 | 440 |
| 3 | 9.5 | 475 |
| 4 | 6.3 | 315 |
| 5 | 6.8 | 340 |
| 6 | 6.0 | 300 |

although the total amount may be the same. This may be obviated to a considerable extent by the use of lecithin, and this has been the method used by most previous investigators.

Lecithin, being a phospholipid, could possibly interfere with the results. Accordingly, in this study the viscosity of the medium was altered by using a 5% solution of recrystallized cholesterol made up in 5% gelatin in 0.9 per cent sterile saline, blended by mixing for a period of 20 minutes in a Waring Blendor.[8] This preserved the suspension of microscopic particles of cholesterol for a much longer period; it could be kept in a solid state in the refrigerator, was inert, and preserved the uniformity of particle size.

The amount given in each injection for the first four weeks was calculated on the basis of 50 mg. per kilogram of body weight. Considering blood plasma volume as approximately 5% of the body weight and using the average of the values recorded during the control period for each dog as approximately the blood plasma cholesterol content, one calculates the above amount roughly as more than 50% of the cholesterol circulating in the blood plasma of the animal. Table 1 gives the total amount of cholesterol injected weekly in each of the six dogs.

The amount was then increased to 75 mg. per kilogram of body weight for the next five weeks. Dog 4 died immediately after the second injection at this level and Dogs 1 and 3 died after five injections. The amount was then dropped back to the 50-mg. level for the remainder of the experimental period.

8. Remond, A.; Columbies, H., and Bernardberg, J.: Compt. rend. Soc. biol. **90**:1029, 1924. Abelous, J. E., and Soula, L. C.: Compt. rend. Soc. biol. **85**:6, 1921.

9. Shillito, F. H.; Bidwell, E. H., and Turner, K. B.: J. Biol. Chem. **112**:551, 1936.

All injections were made in the morning before the dogs had been fed. The animals were maintained throughout the period on a diet which contained 23% protein and 5% fat.[10] A sample of blood for determination of cholesterol was first drawn from the jugular vein. The cholesterol solution was then slowly injected, the same needle being used by leaving it inserted in the vein and substituting the cholesterol syringe for the blood syringe. During the first few weeks of the study a second sample was drawn from 5 to 15 minutes after cholesterol had been injected.

Dogs 2 and 6 were maintained for the six-month period but Dog 5 died at the end of five months after the 22d injection of cholesterol. It was always difficult to draw a blood sample from this animal, and during the fourth and fifth months only two samples could be obtained for determination of cholesterol. Three of the injections had to be made into the vein of the leg instead of the jugular vein.

Dogs 2 and 6 were killed by first injecting pentobarbital sodium and then bleeding. Tissues from Dogs 2, 5, and 6, as well as tissues from 10 dogs not given injections of cholesterol, used as controls, were taken for cholesterol determination. Samples of liver, kidney, adrenal, lung, aorta, heart, and duodenum were used. Slides were also made for histological studies of the tissues.

### METHODS OF DETERMINATION

Plasma cholesterol determinations were made in duplicate by the Liebermann-Burchard reaction using a modification of the Bloor technique.[11] Plasma phospholipids were determined by extracting with alcohol and ether, destroying the organic matter by oxidation with sulfuric acid and hydrogen peroxide, and estimating inorganic phosphorus by a modification of the Fiske and Subbarrow method.[12]

Tissues were dried and moisture content determined. After extraction with an extracting fluid made of equal amounts by volume of alcohol, ether, and acetone, cholesterol was determined by the Liebermann-Burchard reaction.

### RESULTS AND COMMENT

Weekly injections of cholesterol were given to six dogs after a preliminary control period. A blood sample was first drawn for cholesterol and phospholipid determination. During the first few weeks of the study a second sample was drawn after cholesterol had been injected. Determination of blood drawn in as short a period of time as 5 to 15 minutes after injection of cholesterol showed little difference from that drawn before injection. This is in disagreement with the study by Cashin and Moravek.[9] They, however, used lecithin along with cholesterol for injection.

Four of the animals in this study died after injection of the cholesterol solution, three during the period when 75 mg. of cholesterol per kilogram of body weight was being used, and one during the period when the amount was dropped back to the 50-mg. level. In each case death resulted in from 5 to 15 minutes after the injection. The pulse rate and the respirations increased and the dogs died in cardiac arrest.

In each dog, considerable variation was found from one week to the next in plasma cholesterol, cholesterol esters, and phospholipids; there was also variation between animals. When the mean values for the control period of each animal were compared with the mean values for the experimental period no significant differences were found.

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10. This diet was purina® laboratory chow, made by the Purina Ralston Company, St. Louis.
11. Bloor, W. R.: J. Biol. Chem. **24**:227, 1916.
12. Fiske, C. H., and Subbarrow, Y.: J. Biol. Chem. **66**:375, 1925.

Chemical analyses for cholesterol were made on tissues from dogs 2, 5, and 6 and on tissues from the 10 control dogs. Samples of liver, kidney, adrenal, lung, aorta, heart, and duodenum were taken.

The values for moisture content and total cholesterol content of each of the tissues are found in Table 2. Among the control tissues, the largest amounts of cholesterol were found in the adrenal glands, with values ranging from 2.5 to 6.2%. The average percentages for cholesterol found in the other tissues were as follows: kidney, 0.65; lung, 0.63; heart, 0.36; liver, 0.35; duodenum, 0.33; aorta, 0.25.

When the tissues from the experimental animals were compared with similar tissues from the control animals, the averages for cholesterol content of liver, adrenal, heart, and duodenum were found to be slightly higher. In the case of lung,

TABLE 2.—*Moisture and Cholesterol Content of Tissues*

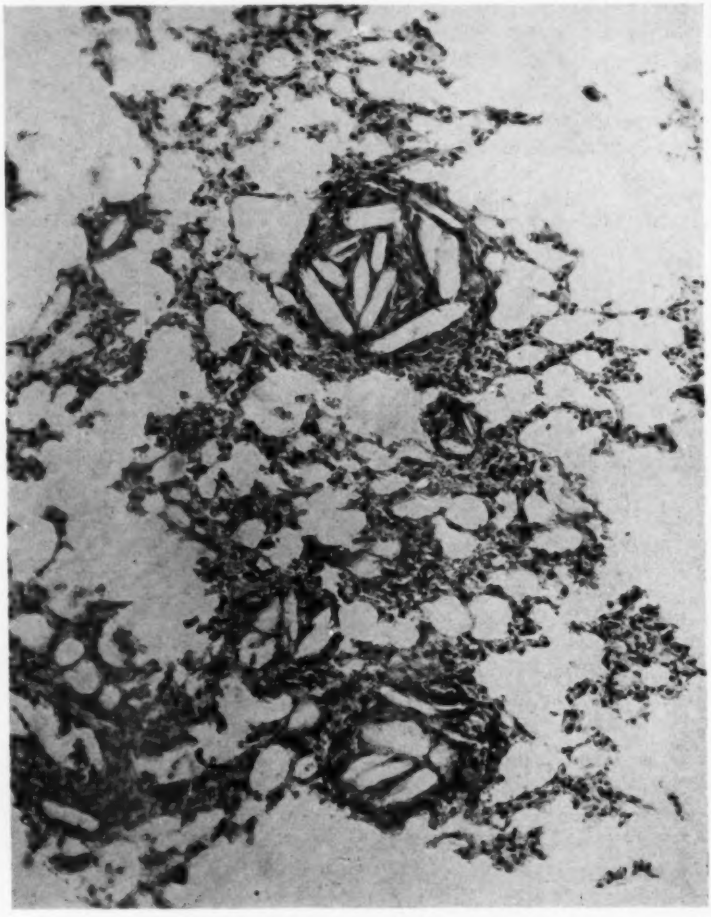
| | Liver | | Kidney | | Adrenal | | Lung | | Aorta | | Heart | | Duodenum | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dog | Moisture, % | Cholesterol, % Dry Wt. | Moisture, % | Cholesterol, % Dry Wt. | Moisture, % | Cholesterol, % Dry Wt. | Moisture, % | Cholesterol, % Dry Wt. | Moisture, % | Cholesterol, % Dry Wt. | Moisture, % | Cholesterol, % Dry Wt. | Moisture, % | Cholesterol, % Dry Wt. |
| Control Dog Tissues | | | | | | | | | | | | | | |
| 1 | .... | ... | 74.8 | .76 | 68.3 | 4.4 | .... | ... | 67.0 | .25 | 75.5 | .42 | .... | ... |
| 2 | 70.2 | .37 | 75.2 | .67 | 64.5 | ... | 75.9 | .56 | 61.1 | .24 | 74.4 | .41 | .... | ... |
| 3 | 70.9 | .33 | 76.8 | .60 | 62.4 | 3.4 | 76.3 | .89 | 62.8 | .28 | 72.7 | .36 | .... | ... |
| 4 | 67.5 | .46 | 74.2 | .54 | 60.9 | 2.5 | 75.6 | .55 | 55.2 | .25 | 72.7 | .28 | .... | ... |
| 5 | 67.7 | .37 | 71.6 | .82 | 51.8 | 4.9 | 72.5 | .49 | 55.3 | .25 | 74.9 | .36 | .... | ... |
| 6 | 66.7 | .39 | 76.3 | .97 | 57.2 | 2.4 | 74.5 | .85 | .... | .25 | 71.6 | .31 | 74.4 | .47 |
| 7 | 65.4 | .33 | 74.1 | .60 | 61.1 | 3.3 | 73.9 | .71 | 59.0 | .23 | 73.6 | .37 | 60.6 | .30 |
| 8 | 64.7 | .33 | 70.8 | .72 | .... | 2.6 | 73.9 | .57 | .... | ... | 71.2 | .36 | 72.8 | .35 |
| 9 | 67.8 | .31 | 74.9 | .44 | 58.5 | 5.9 | 74.9 | .52 | .... | .30 | 74.1 | .44 | 73.5 | .30 |
| 10 | 62.7 | .31 | 73.6 | .44 | 61.9 | 6.2 | 71.3 | .45 | 66.1 | .25 | 71.9 | .30 | 71.2 | .25 |
| 11 | .... | ... | .... | ... | .... | ... | 77.4 | .74 | .... | ... | .... | ... | .... | ... |
| Average | 66.9 | .35 | 74.2 | .65 | 59.6 | 3.9 | 74.6 | .63 | 60.9 | .25 | 73.2 | .36 | 70.5 | .33 |
| Experimental Dog Tissues After Weekly Injections for Five or Six Months | | | | | | | | | | | | | | |
| 2 | 66.8 | .44 | 79.8 | .67 | 55.0 | 6.3 | 74.6 | 4.6 | 63.3 | .21 | 72.8 | .41 | 69.1 | .40 |
| 5 | 68.4 | .40 | 77.1 | .82 | 54.3 | 3.1 | 76.1 | 3.8 | 58.2 | .18 | 75.5 | .41 | 73.0 | .43 |
| 6 | 65.6 | .39 | 74.8 | .88 | 54.3 | 6.1 | 75.4 | 4.0 | 62.2 | .27 | 75.7 | .50 | 71.6 | .46 |
| Average | 66.7 | .41 | 77.2 | .79 | 54.5 | 5.1 | 75.3 | 4.1 | 61.2 | .22 | 74.7 | .44 | 71.2 | .43 |

however, an increase from 0.63% for the control tissues to 4.1% for the experimental tissues was found. When these data were treated statistically, there were no significant differences in any of the tissues except lung, in which the difference was found to be significant at the 0.1% level of confidence. We interpret this as meaning that cholesterol had been deposited in the lung tissues but probably not in any of the other tissues.

All viscera were examined at the time the animals were killed. They were all grossly normal, but on microscopic examination crystalline cholesterol deposits were found in the lung, with cytologic evidence of foreign body reaction. A photomicrograph of lung tissue is shown in the accompanying figure. In no other tissue was there microscopic evidence of cholesterol deposition, and this was to be anticipated as a result of the chemical analyses.

SUMMARY

Six dogs were given intravenous injections of a solution of cholesterol at definite weekly intervals for a period of two to six months. Considerable individual variations in total plasma cholesterol, cholesterol esters, and phospholipids were found from week to week in samples taken before injection, but no significant rise or fall was found in comparing the experimental period with a preliminary control period. No changes were found in blood plasma cholesterol samples taken before and immediately after injection of the solution of cholesterol.



Lung tissues showing crystalline cholesterol deposits.

The cholesterol contents of tissues from 10 dogs used as controls were compared with those of tissues from three of the dogs which had received injections of cholesterol for a period of five to six months.

Analyses of tissues showed that liver, adrenal, heart and duodenum from the animals which had received injections had slightly higher cholesterol contents than control tissues, on comparison of averages, but when the data were treated statistically no significant increases were found. In the case of lung tissue, however, a definite increase had resulted. Histological studies of lung tissues from cholesterol-treated dogs showed deposits of cholesterol. All other histological studies showed normal cholesterol distribution.

The functional significance of these observations, while obviously important, are as yet not clear.

# RUPTURED CONGENITAL ANEURYSMS OF THE AORTIC SINUSES OF VALSALVA

## Report of a Case with Bacterial Endocarditis Due to Hemophilus Parainfluenzae

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CONGENITAL aneurysms of the sinuses of Valsalva are rare. Jones and Langley [1] collected only 23 cases from the literature and added two of their own. The present report is concerned with a case in which two such congenital aneurysms were found. One aneurysm involved the right sinus of Valsalva and had perforated into the pulmonary conus, while the second was situated in the noncoronary sinus and had ruptured into the right atrium. Terminally, endocarditis of the pulmonary valve developed, due to Hemophilus parainfluenzae.

### HISTORY OF CASE

M. B., a 39-year-old male Negro laborer, was referred to the cardiac clinic of the Cincinnati General Hospital on Nov. 19, 1949, for evaluation of a cardiac murmur. Seven months previously, a roentgenologic examination of his chest had been made at another clinic, and he was advised to consult his physician. The findings of this examination were not known. On entering the cardiac clinic he offered no complaints. Except for a vague story of "arm and hip shots," given him several years previously, his past history was without significance.

He was a well-developed and well-nourished Negro man weighing 143 pounds. The pupils reacted to light and accommodation, and vigorous pulsations of the retinal arteries were observed. The heart was enlarged to percussion, and the maximal cardiac impulse was visible in the sixth interspace near the anterior axillary line. Harsh, continuous systolic and diastolic murmurs were audible over the entire precordium but were loudest at a point slightly beneath the pulmonary valvular area. The murmurs were transmitted to the carotid arteries, and the diastolic component was accompanied by a thrill. The pulse rate was 90 and of the Corrigan type. The blood pressure was 120/55 in the right arm and 112/50 in the left arm. There was no edema or venous distention. A few rales were present in the bases of both lungs.

Fluoroscopy demonstrated cardiac enlargement with posterior displacement of the esophagus, forceful cardiac pulsations, and enlargement of the pulmonary arc with increased arterial pulsations in the pulmonary hilums. An electrocardiogram showed a sinus rhythm, PR. 0.16 second, QRS 0.08 second, and flattening of the T waves in Leads 1 and 4. The tracings were interpreted to indicate minimal myocardial damage of nonspecific nature. Urinalysis and a standard Kahn test of the blood gave negative results.

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1. Jones, A. M., and Langley, F. A.: Aortic Sinus Aneurysms, Brit. Heart J. **11**:325-341, 1949.

The patient, considered to be in mild cardiac failure, refused hospitalization and was treated with digitalis as an outpatient. Two months later, moderate pedal edema and a slight gain in weight were observed and were ameliorated by administration of mercurial diuretics.

Under this regimen, the congestive failure was controlled, but lethargy, pallor, and weakness developed, and he was admitted to the hospital March 27, 1950. At this time he was afebrile but appeared chronically ill. The mucous membranes and nail beds were pale. A few rales persisted in both pulmonary bases. The cardiac findings, the pulse, and the blood pressure were essentially the same as when observed in the cardiac clinic. The spleen was palpable 3 finger-breadths beneath the costal margin, and a mass, interpreted as liver, was felt in the epigastrium. The cervical veins were distended but did not pulsate. Cyanosis, clubbing, and petechia were absent.

On admission a hemogram revealed 1,750,000 erythrocytes per cubic millimeter, 6.2 gm. of hemoglobin per 100 cc., and 16,500 leucocytes per cubic millimeter, 85% of which were neutrophils. Subsequently the leucocyte count rose to 27,000 per cubic millimeter and included 72% neutrophils. The urine disclosed albumin (1+) and an occasional erythrocyte and leucocyte in the sediment. These abnormal urinary constituents progressively increased during the course of his illness. A series of tests of liver function, March 31, gave the following values: thymol turbidity, 23 units; cephalin flocculation, 4+ in 24 hours; total lipid, 720 mg. per 100 cc.; serum bilirubin, prompt, 0.45 mg. per 100 cc., and total, 1.2 mg. per 100 cc.; sulfobromophthalein retention, 44%. Serum bilirubin studies, April 12, revealed a prompt reacting level of 7.2 mg. per 100 cc. and a total bilirubin content of 12 mg. per 100 cc. The blood urea nitrogen, which was initially 50 mg. per 100 cc., rose to 105 mg. per 100 cc. shortly before death. A Frei test was negative.

Five of six blood cultures in brain-heart infusion broth yielded a growth of a minute gram-negative bacillus which was considered to belong to the Hemophilus group. H. influenzae typing serum A was not available and typing with H. influenzae serum B was negative. No growth was obtained when X factor alone was provided. The requirement for V factor was not established. In spite of the incomplete data, H. parainfluenzae was considered, on a morphologic basis, to be the most likely organism, since filamentous forms failed to appear on repeated subculture in liquid mediums. Such forms are commonly observed in cultures of H. influenzae.[2] The organism was sensitive to aureomycin in a concentration of 0.2 $\gamma$ per cubic centimeter.

The day after admission the patient's temperature rose to 99.6 F., and during the following ten days it fluctuated between 98 and 102 F. After this period the temperature remained at normal to subnormal levels. He was given blood transfusions and treated symptomatically until the organism from the blood stream was identified. On the 15th hospital day aureomycin therapy was begun and continued daily in a dosage of 1.0 gm. every four hours. Three days later a blood culture was negative. However, the patient failed steadily. Jaundice appeared, uremia progressed, and he died 22 days after being admitted to the hospital. Clinically his death was attributed to bacterial endocarditis superimposed on either a patent ductus arteriosus or syphilitic aortic valvulitis.

## AUTOPSY FINDINGS

The body, that of a well-developed, poorly nourished Negro man, measured 168 cm. in length and weighed an estimated 110 lb. (50 kg.).

The heart was moderately enlarged, weighed 505 gm., and presented the following measurements: tricuspid valve, 135 mm.; pulmonary valve, 110 mm.; mitral valve, 110 mm.; aortic valve, 68 mm.; right ventricular wall, 6 mm.; left ventricular wall, 14 mm. Both ventricles were hypertrophied and dilated, but the enlargement appeared greater on the right. The pulmonary valve and pulmonary conus were markedly dilated.

The cusps of the aortic valve were normal except for a 2-mm. fenestration of the right cusp. An ovoid defect, measuring 3 by 5 mm., was present in the wall of the right sinus of Valsalva between the ostium of the right coronary artery and

2. Wilson, G. S., and Miles, A. A.: Topley and Wilson's Principles of Bacteriology and Immunity, ed 3, Baltimore, Williams & Wilkins Company, 1946, Vol. 1, p. 789.

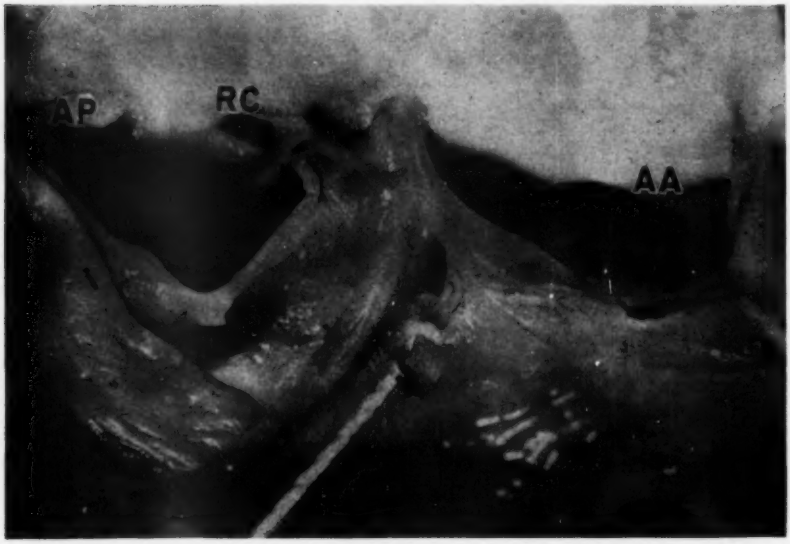


Fig. 1.—Photograph of the aortic valve showing (*AP*) the mouth of the aortopulmonary conal fistula and (*RC*) the ostium of the right coronary artery in the right aortic sinus. *AA*, the orifice of the aortoatrial fistula, is seen in the noncoronary sinus. The rent in the noncoronary aortic cusp is artifactitious.

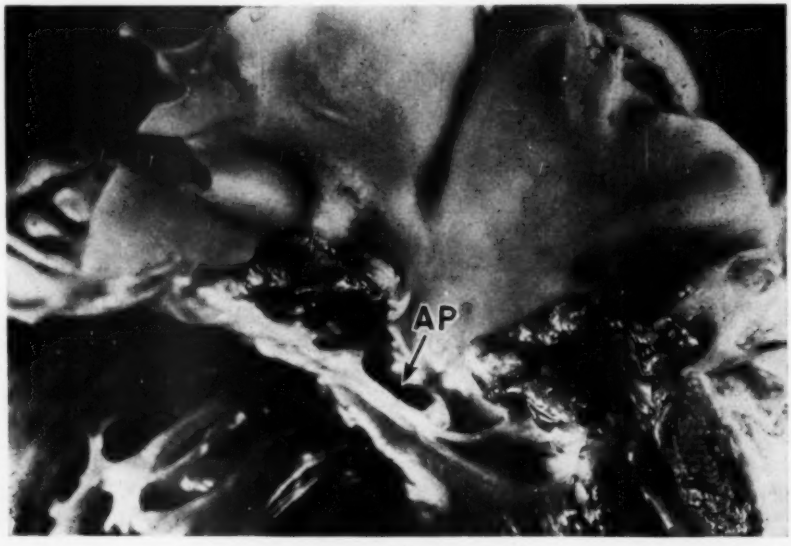


Fig. 2.—Photograph of the pulmonary conus and valve showing (*AP*) the site at which the aortopulmonary conal fistula perforated into the pulmonary conus, and the vegetations on the right and left pulmonary cusps.

the commissure of the right and left cusps (Fig. 1). This defect represented the mouth of a short 5-mm. fistulous tract which entered the adjacent pulmonary conus at the base of the left cusp of the pulmonary valve immediately lateral to the commissure between the right and left pulmonic cusps (Fig. 2). At this point the left cusp was torn from its base and commissure. Bulky, friable, reddish brown vegetations were attached to the ventricular surface of the right and left pulmonary valval cusps, to the intima of the pulmonary artery above the left cusp, and to the endocardium of the pulmonary conus beneath the left cusp.

Another elliptical defect, 3 mm. in diameter, was located in the wall of the noncoronary sinus of Valsalva (Fig. 1). This led into a second fistulous tract which communicated with the right atrium above the commissure of the anterior and septal leaflets of the tricuspid valve (Fig. 3). The right atrial orifice measured

Fig. 3.—Photograph of the right atrium demonstrating (*AA*) the right atrial orifice of the aortoatrial fistula, above the commissure of the anterior and septal leaflets of the tricuspid valve.

4 mm. in diameter and was fringed by minute pink vegetations. The lining of the tract was smooth and continuous with the intima of the aorta.

The left sinus of Valsalva was normal. The heart was lined by a thin, transparent endocardium except for a narrow, 15 mm., transverse, ridge-like, fibrous band which elevated the endocardium beneath the base of the left aortic cusp. The myocardium was uniformly reddish brown and firm. The coronary arteries were normally distributed and patent. The pericardial sac was obliterated by fibrous adhesions.

The lungs weighed 585 and 650 gm., right and left, respectively, and contained multiple small foci of pneumonic consolidation. The tracheobronchial tree contained a thick mucopurulent exudate.

The spleen weighed 535 gm. The pulp was soft and focal hemorrhages were observed in the subcapsular zone.

The liver was slightly enlarged and weighed 1,750 gm. The parenchyma was congested and contained areas of fatty infiltration.

The kidneys weighed 380 gm. together, and the cortices were studded with petechial hemorrhages.

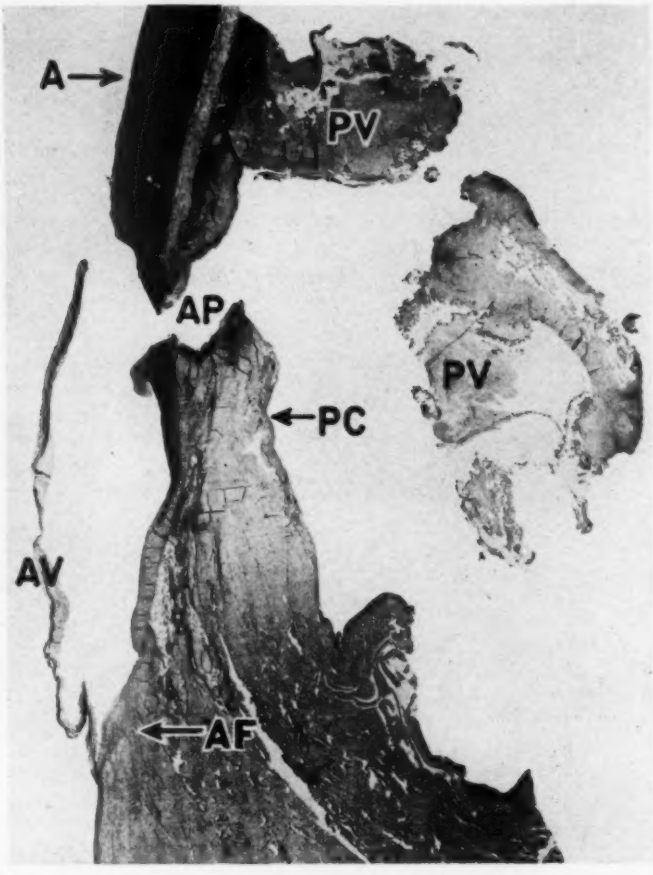


Fig. 4.—Photomicrograph of a longitudinal section of (*A*) aorta and (*PC*) pulmonary conus including (*AV*) the aortic valve, (*AP*) the aortopulmonary conal fistula, and (*PV*) the pulmonary valve. Note the abrupt termination of the aortic medial elastic fibers at the mouth of the fistula, the fanning of the outer elastic fibers into the superior wall of the fistulous tract, and the disappearance of the aortic medial elastic fibers between the mouth of the fistula and (*AF*) the annulus fibrosus. Verhoeff's elastic tissue stain; × 6.

The brain was congested and moderately edematous, and petechial hemorrhages were present in the white matter of the cerebral hemispheres, the brain stem and the cerebellum.

MICROSCOPIC OBSERVATIONS

A longitudinal section through the base of the aorta and pulmonary conus including the aortopulmonary conal fistula (Fig. 4) demonstrated an abrupt termination of the inner medial aortic elastic fibers and a slight fanning of the outer elastic fibers into the superior wall of the fistulous tract. Between the aortic mouth

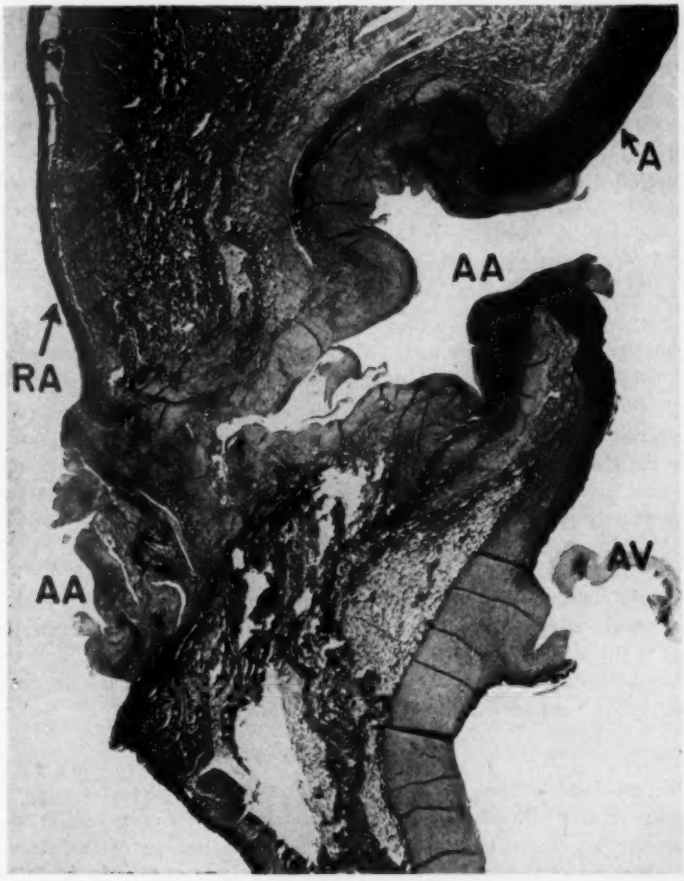


Fig. 5.—Photomicrograph of a longitudinal section of (*A*) aorta, (*AV*) aortic valve, and (*RA*) right atrium, illustrating (*AA*) the aortoatrial fistula. The aortic medial elastica continues intact for a considerable distance in the wall of the aneurysm. Note the normal appearance of the aortic medial elastica in the segment of aorta above the mouth of the fistula. Verhoeff's elastic tissue stain; × 8.

of the fistula and the base of the aortic valve, the medial elastica gradually faded into dense collagenous tissue before reaching the annulus fibrosus. The fistulous tract was endothelized, and its wall was composed of collagenous tissue which merged

with the adventitial coat of the aorta and the connective tissue at the base of the pulmonary valve. At the pulmonary conal orifice there was a slight neutrophilic reaction in the intima.

A longitudinal section through the base of the aorta including the aortoatrial fistula (Fig. 5) revealed a sac-like outpouching of the aortic sinus pointing to the right atrial wall. The elastic fibers of the aortic media continued into the wall of the sac for a distance of 2 mm. At this point the elastic fibers began to fade away, but a few continued almost to the apex of the sac. Here the sac opened into an endothelized tract which entered the right atrium. A slight neutrophilic infiltration was present in the wall of the fistula at the right atrial orifice where the lumen was filled with fresh thrombus.

The left cusp of the pulmonary valve was ulcerated and largely destroyed. It was infiltrated by leucocytes and thickened as a result of connective tissue proliferation. The exuberant vegetation attached to it was composed of fibrin and leucocytes. Calcific deposits were observed in the granulation tissue of the vegetation as well as in the valval cusp. A similar appearance was noted in the other vegetative lesions. The tricuspid, mitral, and aortic valves were normal.

The myocardium was edematous and contained many minute abscesses. These were unusual in that centrally there were remnants of degenerating muscle cells on which small amounts of calcium were deposited. The surrounding inflammatory reaction consisted of a mixture of neutrophils and histiocytes.

Bacteria could not be demonstrated in the pulmonary valvular vegetations or the myocardial abscesses by Glynn's variant of the Gram technic, the Goodpasture, or the Giemsa technic. Postmortem cultures of the blood and of the pulmonary valvular vegetation yielded no growth.

### ANATOMIC DIAGNOSIS

Bacterial endocarditis of the pulmonary valve; congenital aneurysms of the right and the noncoronary aortic sinus of Valsalva perforating into the pulmonary conus and the right atrium, respectively; acute suppurative myocarditis; focal embolic glomerulonephritis; acute suppurative interstitial pancreatitis; acute lobular pneumonia; passive congestion of the liver and the spleen.

### COMMENT

The pathologic findings indicate that a defect in the development of the aortopulmonary septum with subsequent formation and rupture of aneurysms of the right and noncoronary aortic sinuses was the basis for the genesis of the cardioaortic fistulas observed in the present case. This thesis was supported by the demonstration of an orderly lamination of elastic fibers in the proximal wall of the aortoatrial fistula, the slight outward curving of the aortic elastica at the mouth of the aortopulmonary conal fistula, and the defect of elastica in the wall of the right aortic sinus between the mouth of the fistula and the annulus fibrosus. In addition, the orifices of the fistulas were situated at sites where developmental errors are prone to occur, since the right and noncoronary aortic sinuses are developmentally related to the septum which partitions the conus and truncus arteriosus. Finally, factors responsible for

the production of an acquired aneurysm, such as syphilis, trauma, and atherosclerosis, were lacking. The endocarditis was considered a complicating rather than an etiologic factor.

The embryologic basis for the formation of these rare aneurysms is a defective partitioning of the conus and truncus arteriosus in the formation of the bulbar and aortopulmonary septums.[3] Early in the development of the heart, a pair of opposing ridges appear in the walls of the conus and truncus arteriosus, and division occurs as the result of the eventual fusion of these ridges. If fusion is not complete, abnormal communications will persist at various levels, resulting in a bulbar septal defect, a cardioaortic fistula, or an aortopulmonary fistula depending on whether the defect is situated beneath, at, or above the site of development of the aortic valve. If, however, the ridges fuse but consist of tissue of poor texture, aneurysms may evolve as the result of the greater pressure within the left ventricle and the aorta.

The right and noncoronary aortic sinuses and valves develop from local swellings in the ridges which partition the truncus arteriosus, while the left coronary sinus and valve develop from an independent swelling on the opposing wall. In the 25 cases reviewed by Jones and Langley [1] congenital aneurysms were limited to the right and noncoronary aortic sinuses. This distribution is attributed to the fact that the formation of the left aortic sinus is not affected by the aortopulmonary septum.

A cardioaortic fistula is characterized clinically [1] by a harsh, rumbling, continuous murmur accompanied by a thrill, which is usually systolic. The murmur is superficial, simulating a pericardial friction rub, and is heard with maximum intensity in the left third and fourth intercostal spaces. The pulse is of the Corrigan type. Frequently a history of the sudden onset of chest pain accompanied with a substernal vibratory sensation incident to the rupture of the aneurysm is obtained.[3b] The absence of such a history in our case precludes determination of the time of perforation. Electrocardiographic and roentgenographic findings have offered little aid in the diagnosis, but in the present case fluoroscopic examination of the chest revealed markedly increased pulsations of the pulmonary arteries, which was interpreted as indicating a left-right shunt. Cardiac catheterization with determination of the pressure and the oxygen content in the vena cava, the right atrium, the right ventricle, and the pulmonary artery would have been an additional aid in establishing the nature of a left-right shunt. The complicating endocarditis manifested shortly after admission prevented such a procedure in this case. The nature of the murmur, the "hilar dance," the negative serologic test for syphilis, and the subsequent development of endocarditis did, however, lead to a clinical consideration of a patent ductus arteriosus.

Organisms of the Hemophilus group are rarely a cause of bacterial endocarditis. Thayer [4] encountered this group of organisms in 2.94% of a series of 306 cases of

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3. (*a*) Kramer, T. C.: Partitioning of Truncus and Conus and Formation of Membranous Portion of Interventricular Septum in Human Heart, Am. J. Anat. **71**:343-370, 1942. (*b*) Abbott, M. E.: Clinical and Developmental Study of a Case of Ruptured Aneurysm of the Right Anterior Aortic Sinus of Valsalva Leading to Communication Between the Aorta and the Base of the Right Ventricle, Diagnosed During Life; Opening in Anterior Interventricular Septum; Malignant Endocarditis, Contrib. Med. & Biol. Research. (Osler Memorial), New York, Paul B. Hoeber, Inc., 1919, Vol. 2, pp. 899-914. (*c*) Patten, B. M.: Human Embryology, Philadelphia, The Blakiston Company, 1947, pp. 666-671. (*d*) Jones and Langley.[1]

4. Thayer, W. S.: Bacterial or Infective Endocarditis, Edinburgh M. J. **38**:237, 1931; ibid. **38**:307, 1931.

bacterial endocarditis. Craven, Poston, and Orgain [5] found only 34 cases of endocarditis due to organisms of the Hemophilus group recorded in the literature and added two cases of their own. Their second case was the only one in which involvement was limited to the pulmonary valve. Factors predisposing the pulmonary valve to endocarditis were absent in their case. In our case the preexisting cardioaortic fistulas may well have set the stage for the subsequent development of endocarditis.

Goetz and Peterson [6] stated that only nine cases of recovery from bacterial endocarditis due to gram-negative bacilli have been recorded in the literature. In six of these the organism belonged to the Hemophilus group, and these authors added two cases in which H. influenzae was the offending organism. Streptomycin, alone or in conjunction with penicillin, was instrumental in recovery in four of these cases, but the therapy employed in the remaining four is not stated. In our case the demonstrated sensitivity of the organism to aureomycin, and the sterile cultures of the blood and of the pulmonary valvular vegetation obtained at autopsy suggest that the newer antibiotics may prove efficacious in the treatment of endocarditis caused by Hemophilus organisms.

SUMMARY

A case of congenital aneurysms involving both the right and the noncoronary aortic sinus of Valsalva and perforating into the pulmonary conus and the right atrium, respectively, is presented. Terminally bacterial endocarditis developed, involving the pulmonary valve, due to Hemophilus parainfluenzae.

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Department of Pathology, Wesley Memorial Hospital, Chicago (Dr. Tenczar).

Department of Electrocardiography, University of California Hospital, San Francisco (Dr. Kaufman).

5. Craven, E. B., Jr.; Poston, M. A., and Orgain, E. S.: Hemophilus Para-Influenzae Endocarditis, Am. Heart J. **19**:434-454, 1940.

6. Goetz, F. C., and Peterson, E. W.: Endocarditis Due to Hemophilus Influenzae, Am. J. Med. **7**:274-279, 1949.

# Case Reports

## MALIGNANT TERATOMA OF THE HEART

### Report of Case with Necropsy

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PRIMARY malignant tumor of the heart in infancy is noteworthy for its rarity; only three cases have been listed in the detailed review of cardiac tumors by Mahaim [1] and in the recent paper dealing with primary cancer of the heart by Whorton.[2] Apparently no malignant teratoma arising in the heart has been reported, nor do the tumors of the heart described as "pleomorphic" or "embryonic" meet the requirements for recognition as primary teratomas, either in the written accounts or in the photomicrographs. In the case which forms the subject of this report the diagnosis of teratoma was made during life from a biopsy of a huge subcutaneous metastasis. That this originated in the heart was not established until the time of the necropsy.

The close resemblance between certain cystic structures found in this highly malignant tumor and the occasionally described epithelial cysts of the heart [3] provided especial interest in this case and suggested a clue to its origin.

#### HISTORY OF CASE

A 2-year-old girl was admitted to the pediatric service of the Research and Educational Hospitals of the University of Illinois for treatment of a right supraorbital tumor, which was first noticed six weeks previously. The child's birth and early development had been normal. Two grandparents had died of cancer.

Attention was called to the supraorbital lesion after a fall in which she had struck her forehead. A bruise appeared, and this receded in three to four days. One week after the initial injury she fell again, traumatizing the same region of the head. Subsequently a swelling appeared, and this gradually increased in size. Believing it to be a hematoma, the child's physician made unsuccessful attempts to withdraw fluid by needle aspiration. One month after its appearance, a biopsy was taken from the tumor, which had grown to the size of a golf ball. The diagnosis of sarcoma was made at another hospital, and it was believed that the tumor above the eye represented a metastasis.

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Dr. Solomon is a former trainee of the National Cancer Institute.

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From the Department of Pathology, University of Illinois College of Medicine.

1. Mahaim, I.: Les tumerus et les polypes du coeur, Paris, Masson & Cie, 1945.

2. Whorton, C. M.: Cancer **2**:245, 1949.

3. (*a*) Stoekenius, W.: Zentralbl. Herz u. Gefässkr. **11**:73 and 89, 1919. (*b*) Kolatschow, A.: Zentralbl. allg. Path. **57**:310, 1933. (*c*) de Chatel, A.: Frankfurt. Ztschr. Path. **44**:426, 1933. (*d*) Davidsohn, I.: Epithelial Cyst of the Heart, Arch. Path. **26**:422, 1938. (*e*) Rezek, P.: Arch. f. path. Anat. **311**:305, 1938. (*f*) Anderson, W. A. D., and Dmytryk, E. T.: Am. J. Path. **22**:337, 1949. (*g*) Rabson, S. M., and Thill, L. J.: Am. J. Path. **24**:655, 1948. (*h*) Leighton, J.; Hurst, J. W., and Crawford, J. D.: Squamous Epithelial Cysts in the Heart of an Infant with Coincident Cystic Changes in the Ovaries and Breasts, Arch. Path. **50**:632 (Nov.) 1950.

On initial examination at the Research and Educational Hospitals the tumor measured 4 by 3 by 3 cm., was firm, nonfluctuant, and fixed to the right frontal bone. Roentgenological examination of the skull revealed some destruction of bone beneath the soft tissue swelling, and a chest roentgenogram showed a roughly spherical mass in the right anterior region of the mediastinum.

A review of the slide from the original biopsy showed the tumor to consist chiefly of gland-like spaces lined by rather uniform rounded cells having the appearance of epithelium. The intervening stroma had a myxomatous appearance produced by sparsely distributed, poorly differentiated cells lying within a loose-textured, pale-staining matrix. Although multigerm layer origin could not be demonstrated in the small fragment available for examination, it was felt that the immaturity of the structures was compatible with a diagnosis of teratoma. The mediastinal mass demonstrated by roentgenogram was believed to represent the primary tumor.

Growth of both the tumor of the head and that of the mediastinum was spectacular during the patient's three-month hospital course, which led to her death. This growth occurred despite vigorous treatment with x-rays. An anemia and granulocytopenia set in, possibly as a result of the therapeutic efforts; they responded favorably to transfusions of whole blood. During the last few weeks of the child's life the manifestations of a superior vena cava syndrome were present. Respirations became increasingly labored prior to death.

## NECROPSY

At autopsy (six hours after death) the body showed some wasting, but physical development was good. One's attention was immediately drawn to a tremendous mass projecting from the right orbitofrontal region of the skull and covered by taut skin (Fig. 1). This tumor, lobulated and rather soft in consistency, measured 26 cm. in greatest diameter. It was fixed to the skull and palpably penetrated through the cranial bones; the right orbit was totally occupied by the tumor. The globe of the right eye had disappeared, although at a distance below its accustomed place, the greatly distorted lids could still be recognized. The tumor extended as far medially as the inner canthus of the left eye and as far posteriorly as the lambdoidal suture, the edema of the scalp reaching to the occiput. Cranial sutures were separated over the vertex, and the fontanels were wide apart. The right naris was reduced to a slit, but there was no invasion of the nasal or the oral cavities. The overlying skin was ulcerated, and in several places the mass was fluctuant.

General inspection revealed the body to be covered with an excessive growth of hair.

When the peritoneal cavity was opened, the edge of the liver was found to lie 6 cm. below the costal margin on the right and 3 cm. below on the left. No abnormality was noted in the pleural spaces, but fine adhesions were found to bind the leaves of the pericardium at all points. These were broken readily, and with the exposure of the pericardial cavity, a greatly distorted heart was seen (Fig. 2). Projecting from its anterior surface in the region of the right auricle was a pinkish tan, lobulated mass, extending anteriorly and laterally over the great vessels, apparently covered by epicardium. Dissection of the heart demonstrated a solid tumor taking origin within the interauricular septum and projecting into the cavity of the right auricle. The endocardium overlying the mass was not eroded and there was no adherent thrombus. Constriction of the right auricle as well as compression of the superior vena cava had occurred. There was a 3-mm. patency of the foramen ovale. Noteworthy was the right ventricular hypertrophy. The heart valves were thin and delicate. The pulmonary artery and its branches lay

free of the tumor, but the first 2 cm. of the aorta was partially enclosed in the tumor, which had reduced somewhat the caliber of the vessel. The coronary sinus and its tributaries and also the coronary arteries were uninvolved.

When sectioned, the tumor was found to consist of heterogeneous tissue: white and yellow, soft, pultaceous or cystic tissue. There were some gelatinous, trans-

Fig. 1.—Large metastatic tumor projecting from right orbitofrontal region of the skull.

lucent areas and a number of larger cystic spaces at the lesion's center, the latter structures measuring as much as 1.0 cm. in diameter.

The lungs were studded with nodules of tumor tissue, chiefly peripheral in distribution, varying in size from 1.0 to 3.0 cm. The nodules bulged from the parenchymal surfaces and were moderately firm in consistency. Sectioned surfaces consisted of a variegated yellow and tan, glistening, fleshy tissue. Elsewhere in the lungs were regions of pneumonic consolidation.

The liver revealed a rounded anterior margin and yellowish color from excessive fat. The remaining abdominal and pelvic organs were normal. Neoplastic tissue was not found in any area below the level of the diaphragm.

The large mass attached to the skull proved to be sharply delineated from surrounding structures. It had eroded the right frontal bone and entered the cranial cavity through a ragged 5.0-cm.-wide defect. Into the right frontal lobe of the brain projected a fairly smooth, globular tumor, which in part had forced the dura ahead of it, but which had also invaded and destroyed a portion of the

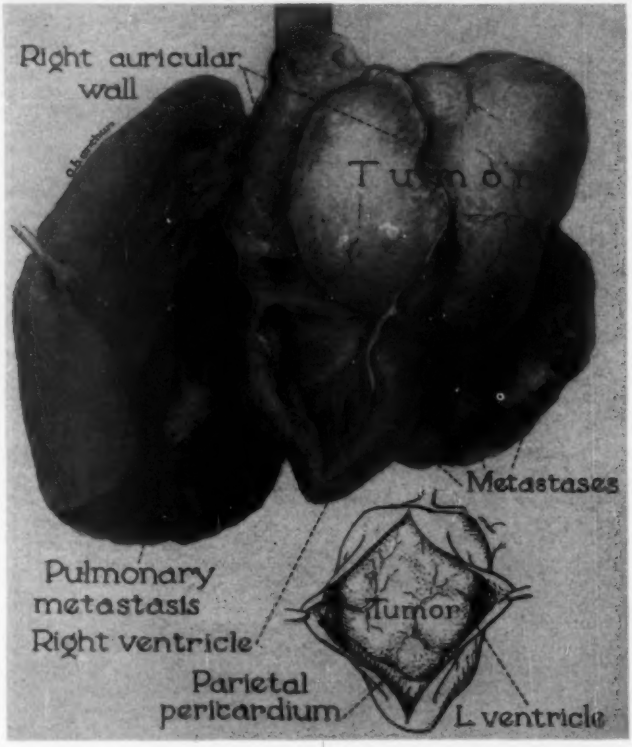


Fig. 2.—Drawing of the heart and the lung showing the primary tumor in relation to the heart and pericardium and the metastases in the lungs.

intervening dura. The brain was not grossly invaded, but there were, of course, much distortion and some necrosis of the frontal lobe, as well as softenings of the right occipital pole, due to pressure. The right cerebral hemisphere had herniated through the falx, and the right lateral ventricle was reduced to a narrow slit. The opposite hemisphere was edematous and its ventricular system dilated.

The nasal and ethmoid bones were intact, and the antrum appeared to be free of tumor. The globe of the eye was located beneath the massive tumor, which had displaced it medially and inferiorly.

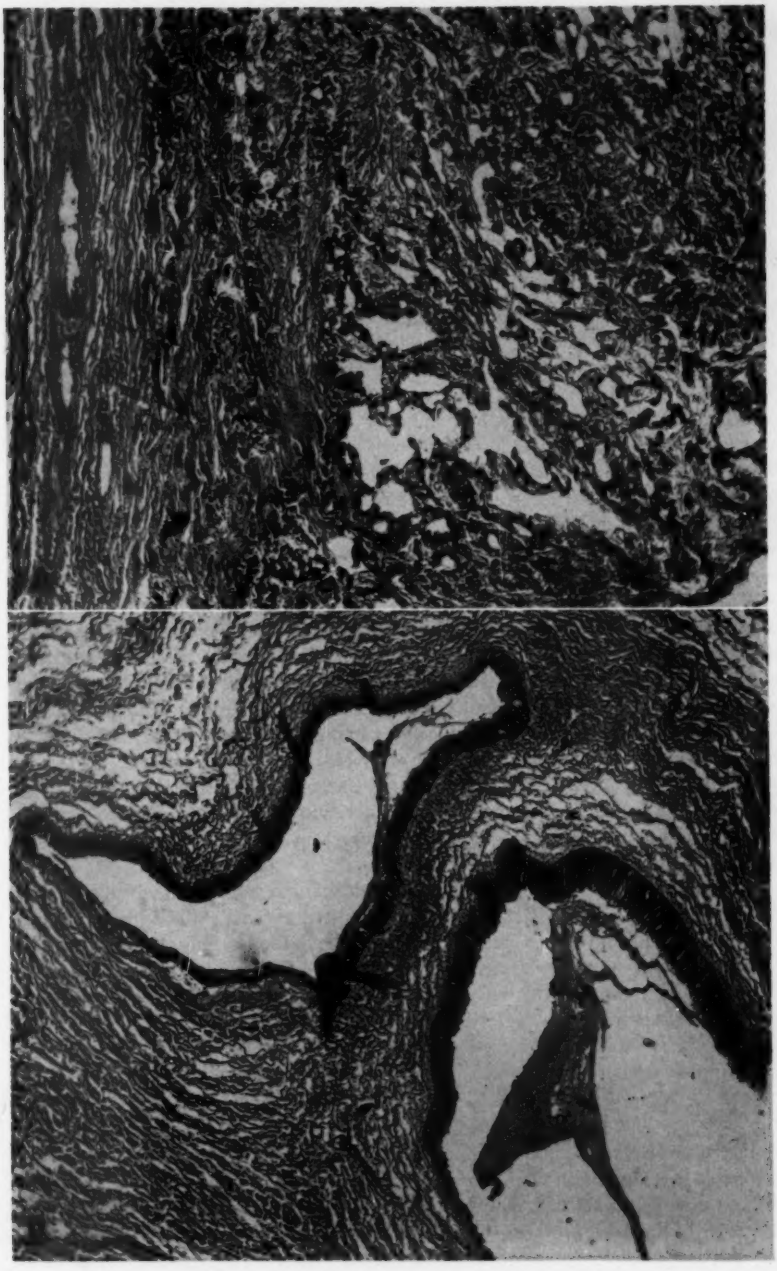

Fig. 3.—*A*, a margin of the tumor illustrating the predominant microscopic features of the primary neoplasm and its metastases. Hematoxylin and eosin stain; × 112.

*B*, cystic spaces in the intracardiac neoplasm, which are lined in some areas with cylindrical epithelium and in other areas with squamous epithelium, are illustrated in this reproduction. Hematoxylin and eosin stain; × 112.

When dissected free, the principal tumor was found to weigh 2,100 gm. Its cut surfaces resembled quite closely those surfaces in the many lung nodules save for the presence of more prominent areas of hemorrhage. Cystic structures like those found in the cardiac tumor were not observed.

### MICROSCOPIC EXAMINATION

Sections taken through the wall of the right auricle established that tumor tissue was present within the myocardium (Fig. 3*A*). This growth had a teratomatous appearance, including a variety of structures. The bulk of the tumor was made up of tiny pseudoglands lined with a single layer of poorly differentiated cells having the appearance of epithelium. These cells had large nuclei with prominent, though variable, chromatin masses. The scant cytoplasm was basophilic, and there was evidence of secretory activity. These small cysts or pseudoglands lay in compact groups amid a spare, largely basophilic or transparent supporting tissue. This poorly developed myxomatous stroma consisted of few, widely spaced, primitive cells and endothelium-lined, thin-walled blood vessels lying within the basophilic and sparsely fibrillated intercellular matrix. There were few argentophilic fibers demonstrable within this stroma. In almost every section there were necrotic portions and partly calcified areas. There was no clearly defined cartilage and no osseous tissue.

The histological appearances of the intracranial tumor and the pulmonary nodules were identical. The cuboidal cells forming pseudoglands constituted the basic structural pattern. The nuclei of these cells, which resembled epithelium, were hyperchromatic, although anisocytosis and anisonucleosis were not striking features. Mitoses were infrequent. Lying in the basophilic intercellular substance were a few scattered mesenchymal cells, responsible for the loose, myxomatous felt-work supporting the tumors. There were also areas of hemorrhage, and in the case of the cranial tumor, much necrosis. Careful search revealed no cysts, no respiratory or squamous epithelium, or other adult structures. There were no features of the microscopic appearance of the cranial tumor to explain its rapid, destructive growth.

Thus the elements which predominated in the cardiac tumor and which composed the entire bulk of the metastatic tumors consisted of small gland-like structures embedded in a primitive myxomatous stroma. In addition to this element, however, the tumor of the heart contained other structures not found in the massive tumor of the head or in those of the lungs. In the cardiac tumor there were various larger cysts lined either with a well-developed ciliated columnar epithelium of respiratory type or with a stratified squamous epithelium showing intercellular bridges (Fig. 3*B*). In one section a mass of cells resembling sebaceous glands was identified.

### COMMENT

That the primary tumor in this case was the one growing in the heart was a reasonable assumption on clinical grounds; it was already of considerable size when the first roentgenogram of the chest was made. It was evident thereafter that the tumor of the head grew with greater rapidity. The cardiac origin of the tumor also was indicated by microscopic examination. Only in the cardiac tumor were respiratory and squamous epithelium and sebaceous glands observed. Essentially, then, only a single element of the teratoma metastasized, namely, the alveolar structures or pseudoglands.

Willis [4] has recently reviewed the literature with reference to intrapericardial teratomas and reported a case of his own. He found descriptions of four cases of benign cystic teratoma; three of the patients were infants, and the fourth was a boy of 14; Willis' patient was an infant of 10 months. The tumors were attached by pedicles to the adventitia of the great vessels and, therefore, unlike the present one, did not arise from within the myocardium. They were considered rather to be examples of mediastinal teratomas, in which the main course of growth had been downward into the pericardial sac.

De Chatel,[3c] some years ago, described a tumor, apparently similar to the one presented here, which had arisen from the interauricular septum of a newborn girl. Within the tumor were two cysts, one lined with keratinizing squamous epithelium and the other with tall columnar cells. About these cysts were alveolar structures, which he likened to sweat glands. He regarded this as a dermoid cyst of the heart. It was very likely a teratomatous tumor and apparently benign.

A striking feature of the tumor considered here is the histological resemblance it bears, on the one hand, to the rare epithelial cysts of the heart, and, on the other, to the mesotheliomas of the pericardium. It is curious that in this case the epithelial cysts occurred in the primary tumor only. No instance of cancer in association with the presence of such gland-like structures and cardiac cysts has been reported, although in the case studied by Anderson and Dmytryk [3f] there was evidence of local invasion. Their patient was a 53-year-old woman who came to the hospital with signs of congestive heart failure. One week later, when a postmortem examination was performed, an egg-shaped tumor, measuring 8 by 5 cm., was found to lie within the wall of the right atrium. Microscopically the tumor exhibited a myxomatous structure in some areas and a densely cellular sarcomatous appearance in other areas. Pseudoglandular formations were found throughout the mass, these being lined with cells ranging from low-cuboidal to tall-columnar. The authors commented that there was a suggestion of cilia issuing from some of the cells but that there was no certainty of their presence. That the growth was malignant was evident locally in the tumor's invasion of the myocardium and also in its invasion of epicardial fat, but there was no metastasis.

The similarity of this tumor to mesotheliomas, or celotheliomas, of the pericardium is less substantial, but there were distinct histological resemblances. To be sure, in mesotheliomas there is a tendency toward generalized involvement of the serous surfaces by sheetlike extensions or nodular thickenings. Furthermore, the type of metastatic growth in the present case would not be expected of a mesothelioma. An example of such a tumor which was given by Dick [5] is of interest in this connection. His patient, a 21-year-old man, died of congestive heart failure and pericardial effusion. Postmortem examination disclosed a nodular thickening of the entire visceral pericardium, involving particularly the superior vena cava and the pulmonary artery. The parietal pericardium contained a few nodules of tumor, and there was extension to the mediastinum and the root of the lung. Histologically, the tumor consisted chiefly of small alveolar structures. Round or elongated acini were lined with cells resembling epithelium.

4. Willis, R. A.: J. Path. & Bact. **58**:284, 1946.
5. Dick, J. G.: J. Path. & Bact. **47**:43, 1938.

Other authors have not mentioned this alveolar arrangement in reports of mesotheliomas of the pericardium, although this has been a prominent feature of tumors identified as mesotheliomas arising in pleura or peritoneum.

Whatever the origin of the epithelial cysts of the heart, whether from pericardial or from esophageal inclusions or from heterotopia, as has been suggested, it is likely that the tumor in this 2-year-old girl had a similar genesis. In view of the frequency of mediastinal teratomas it is assumed that multipotential cells of the mediastinum may be included within the developing heart. The occurrence of a tumor like the present one suggests the possibility that there is a relationship between epithelial cysts of the heart and mediastinal teratomas.

SUMMARY

A case of malignant teratoma arising in the right cardiac auricle of a 2-year-old girl is presented. The similarity between this tumor and the so-called epithelial cysts of the heart is pointed out, and a relationship to mediastinal teratomas is suggested.

# THYMIC CYST OF THE NECK

RUSSELL W. WELLER, M.D.
ALEXANDER E. PEARCE, M.D.
AND
MILTON RAPOPORT, M.D.
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THYMIC cysts of the mediastinum have received little or no attention in most textbooks of pathology. They are rare in adults, usually small, and usually found at autopsy. A few reported occurred in infants and children. Their occurrence in the cervical region, however, is even less commonly recorded. We are able to find only two previous reports of thymic cysts situated unilaterally in the upper cervical region. Hyde, Sellers, and Owen [1] described a thymic cyst below the angle of the right jaw in a 5-year-old white boy. It appeared eight months preoperatively as a bulge in the neck, and gradually increased in size. At operation the cyst extended to the pharynx and along the anterior surface of the common carotid artery to below its bifurcation. Heinz [2] described a "branchial cyst" with abundant lymphoid stroma containing Hassall's corpuscles, and cystic spaces with papillary projections lined with stratified squamous epithelium.

We feel that the following case is worthy of reporting for two principal reasons: (1) the necessity for considering thymic cyst in the differential diagnosis of a cystic lesion of the neck; (2) the additional information it may contribute concerning the pathogenesis of thymic cysts.

## CLINICAL OBSERVATIONS

A 4½-year-old white girl (history No. 57409) was admitted to Hahnemann Hospital on June 22, 1951, with a chief complaint of swelling in the left side of the neck of four months' duration. Her mother at first noted a small, tender mass, which grew steadily in size. Physical examination revealed no abnormality except for an ovoid cystic mass lying beneath the sternocleidomastoid muscle from the angle of the jaw to the clavicle. The mass extended from 2 cm. lateral to the midline in front to the edge of the trapezius muscle behind. It was not tender.

The laboratory findings were not remarkable.

The initial impression was left branchial cyst.

The next day, with the child under endotracheal nitrous oxide-oxygen-ether anesthesia, excision was performed (A. E. P.) A transverse incision over the middle of the sternocleidomastoid muscle was made. The muscle was carefully retracted posteriorly and the cyst exposed (Fig. 1). It had grown into the carotid sheath and was closely adherent to the internal jugular vein and the carotid arteries; it encircled the vagus nerve, which was displaced

Assistant Professor, Department of Pathology (Dr. Weller), Associate Professor, Department of Surgery, Hahnemann Medical College and Hospital (Dr. Pearce), and Associate Professor, Department of Pediatrics, University of Pennsylvania School of Medicine (Dr. Rapoport).

1. Hyde, T. L.; Sellers, E. D., and Owen, M.: Thymic Cyst of the Neck: Report of a Case, Texas J. Med. **39**:539, 1944.

2. Heinz, I. C., in King, E. S. J.; Lowe, T. E., and Cox, L. B.: Studies in Pathology, Presented to Peter McCallum, Carlton, Victoria, Australia, Melbourne University Press, 1950, p. 69.

posteriorly almost to the edge of the trapezius muscle. Posteriorly the tumor extended to the prevertebral musculature. Superiorly it lay against the parotid gland at the angle of the mandible. Inferiorly it lay on the pleural apex. No connection with the pharynx was demonstrated. The cyst was excised intact, bleeding points were controlled with fine-silk sutures, and the wound was closed intact with fine silk; a tiny drain was brought out the posterior angle.

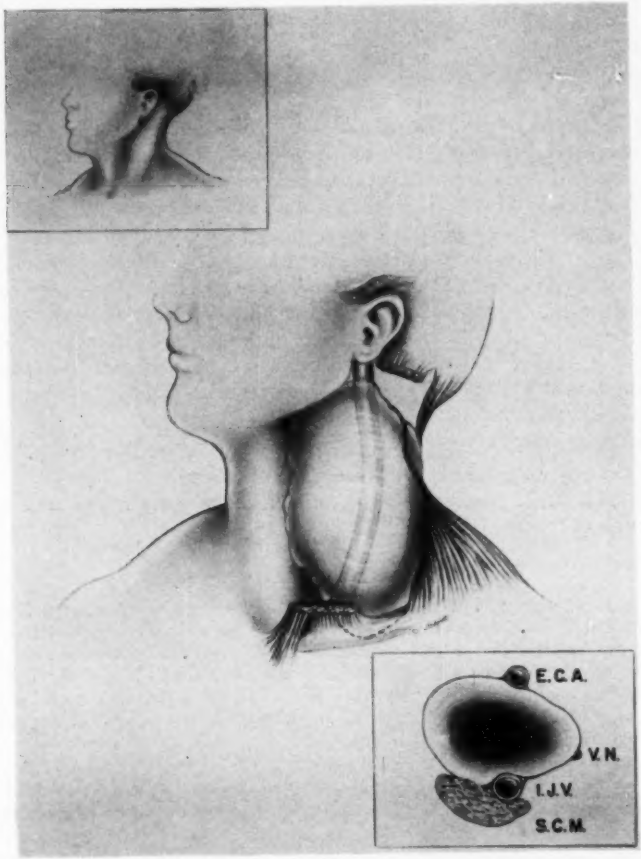


Fig. 1.—Sketch of the neck showing the location of the cyst before surgical exposure (inset above), after surgical exposure (middle), and on cross section, with relation to the external carotid artery (*E. C. A.*), the vagus nerve (*V. N.*), the internal jugular vein (*I. J. V.*), and the sternocleidomastoid muscle (*S. C. M.*).

The patient made an uneventful recovery and was discharged on June 25. The sutures and the drain were removed on June 27.

## PATHOLOGICAL OBSERVATIONS

Grossly the specimen consisted of a unilocular cyst measuring 4.0 by 7.0 by 4.5 cm. The wall was made up of pinkish-gray tissue and varied from 0.1 to 0.5 cm. in thickness. The cyst contained cloudy, pale brown fluid. The inner lining was pink to

pinkish brown and in the thickened areas presented small nodules up to 0.4 cm. in diameter which projected into the lumen.

Microscopically the cyst was lined by flattened cells in the thinner portions and by stratified squamous epithelium in the thicker, nodular portions, which had fibrous cores and projected for short distances into the lumen. On the epithelial surface focal collections of monocytes, plasma cells, lymphocytes, and foreign-body giant cells were noted (Fig. 2). The wall of the cyst was made up of fibrous tissue in

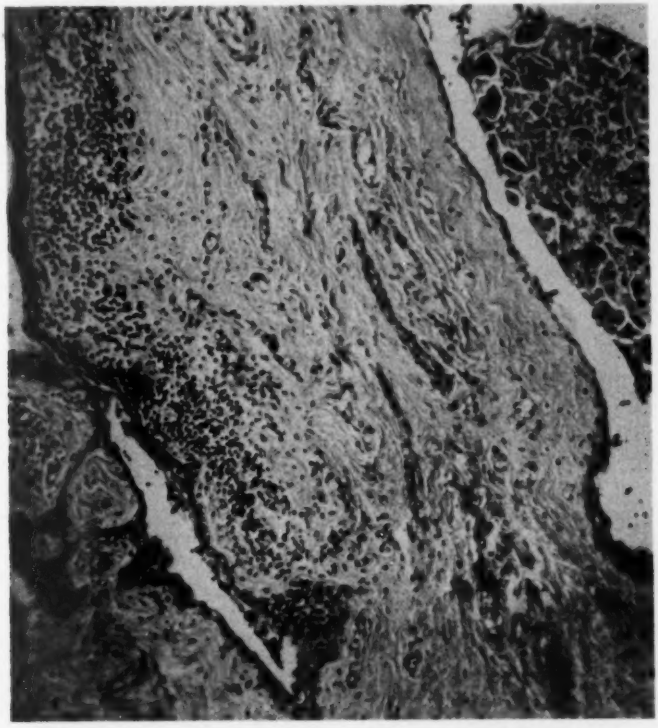

Fig. 2.—Wall of the cyst showing a nodular structure protruding into the lumen and, at upper right, a section of granulation tissue containing foreign-body giant cells. Hematoxylin and eosin stain; × 100.

which there were many focal collections of lymphocytes and occasional Hassall's corpuscles, some of which had necrotic centers (Figs. 3 and 4). Occasional cholesterol clefts surrounded by foreign-body giant cells and mononuclear phagocytes were present in the cyst wall.

## COMMENT

Speers [3] stated that thymic cysts may develop in any one of the following ways: (1) from embryonal remnants of branchial clefts, thymic tubules, or thymopharyn-

3. Speers, F. D.: Thymic Cysts: Report of a Thymus Presenting Cysts of 3 Types, New York M. Coll. & Flower Hosp. Bull. **1**:142, 1938.

geal ducts; (2) from sequestration products in pathological involution of the thymus; (3) from neoplastic processes in the lymphoid, cytoreticular, or connective tissues; (4) from degenerating Hassall's corpuscles; (5) from lymph vessels, blood vessels, or connective tissue in various stages of thymic development, hyperplasia, or involution.

The cyst described here seems not to be neoplastic but rather to be an embryonal remnant of a branchial cleft or thymopharyngeal duct which, owing to inflammation of unknown cause, became cystically dilated and clinically noticeable. When the development of the human thymus from the third branchial pouch and its descent

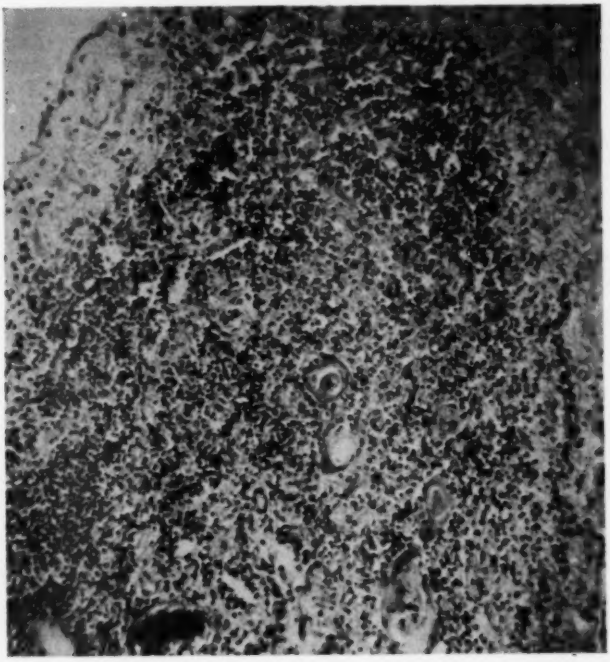

Fig. 3.—Wall of cyst with flattened cell lining and in the deeper portions lymphocytes and Hassall's corpuscles. Hematoxylin and eosin stain; × 100.

from the neck are considered, one ponders why thymic lesions are not more frequently described in the neck, particularly in branchial cysts. It is not uncommon for a bit of thymic tissue to remain associated with a parathyroid gland.

We are unable to state whether the age of this patient is in any way related to the pathogenesis of the disease. Bradford [4] cited several authors who believed thymic cysts in infants were usually secondary to congenital syphilis. Speers [5] noted thymic

4. Bradford, M. L.; Mahon, H. W., and Grow, J. B.: Mediastinal Cysts and Tumors, Surg., Gynec. & Obst. **85**:467, 1947.

cysts at autopsy in a case of lead poisoning. Plagge [5] produced extensive small cysts of the thymus, particularly at the cranial extremity of the organ, in rats by prolonged, massive treatment with estradiol dipropionate.

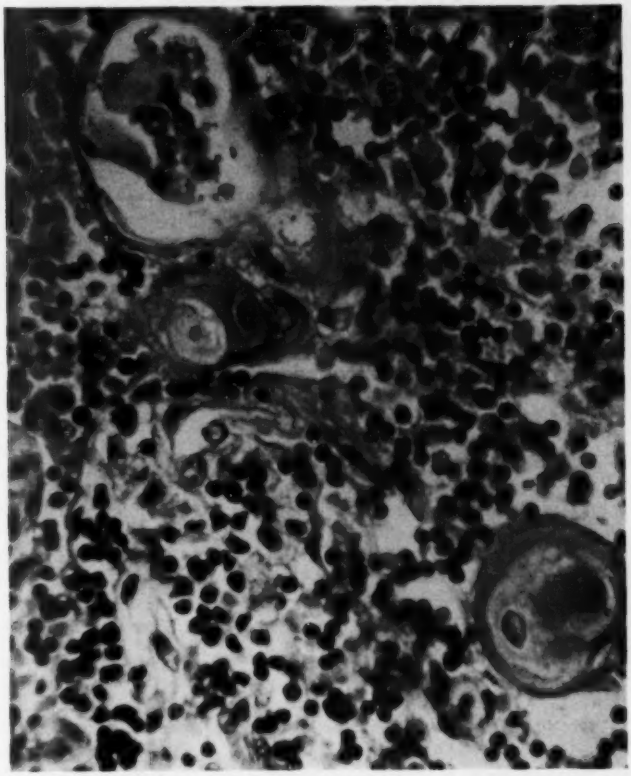

Fig. 4.—Several Hassall's corpuscles. The one in the upper left corner has necrotic center. Hematoxylin and eosin stain; × 430.

SUMMARY

A thymic cyst situated unilaterally in the neck of a 4½-year-old girl is described. The lesion seems to be extremely rare but should be considered in the differential diagnosis of cystic lesions of the neck. The pathogenesis of thymic cysts is discussed, and it is concluded that the cyst reported here most likely developed from an embryonal remnant of a branchial cleft or a thymopharyngeal duct.

5. Plagge, J. C.: Some Effects of Prolonged Massive Estrogen Treatment on the Rat, with Special Reference to the Thymus, Arch. Path. **42**:598, 1946.

# News and Comment

## STANDARD NOMENCLATURE OF DISEASES AND OPERATIONS

**Fourth Edition**

The American Medical Association announces the publication of the Fourth Edition of "Standard Nomenclature of Diseases and Operations." Changes in the new edition have been as much as possible kept to a minimum. Nevertheless, advancing knowledge has made necessary a complete revision of the section on the psychobiologic unit, diseases of the hemic and lymphatic systems, and tumor etiology. A completely new feature of this edition is the inclusion of code numbers of the International Statistical Classification of Diseases, Injuries, and Causes of Death, parenthetically in the body of the book, and a cross reference of International and Standard code numbers as an appendix. The new edition is being published for the Association by The Blakiston Company (Philadelphia) and will be available Jan. 2, 1952.

# Books Received

DIBLE AND DAVIE'S PATHOLOGY: AN INTRODUCTION TO MEDICINE AND SURGERY. By J. Henry Dible, M.B (Glas.), F.R.C.P. (Lond.), professor of pathology, University of London (Post-Graduate Medical School of London); late professor of pathology, London School of Medicine for Women; professor of pathology, Welsh National School of Medicine, and professor of pathology, University of Liverpool. Third edition. Pp. 963, with 417 illustrations, including 9 plates in color. Price $13. J. B. Lippincott Company, 227-231 S. Sixth St., Philadelphia 5, 1951.

The reviewer first became acquainted with this textbook of pathology during the early years of World War II when in the moments that could be spared from Army duty he assisted with the course in pathology in the University of Melbourne. Since that time the book has been twice revised, most recently by Professor Dible alone, as professor Davie has assumed administration duties at the University of the Cape.

This book was written as a textbook for students with the purpose of giving them the essentials of pathology so that they might understand clearly the natural progress of disease before undertaking their clinical work. In this purpose the authors have achieved success, for the principles of pathology are set forth logically and clearly. Important diseases are stressed at the expense of unimportant ones, and many rare diseases are intentionally omitted, for the authors have not attempted to write a book which is a "repository of information upon all and every sort of pathological condition." Although the needs of the medical student have been given priority, graduate and professional students of pathology will also profit greatly from a reading of the many fine sections in the book, such as those on "Disordered Fat Metabolism," "Diseases of the Endocrine Glands," "Diseases of the Liver," and "Kidney"—to mention only a few. These are clear, logical reviews of the diseases under discussion which are very stimulating indeed.

The lack of a bibliography is disappointing, and no doubt the author will revise the section on tumors in the light of recent experimental investigations. The pleasing format of the book and the excellence of the illustrations add greatly to the enjoyment of this excellent textbook of pathology.

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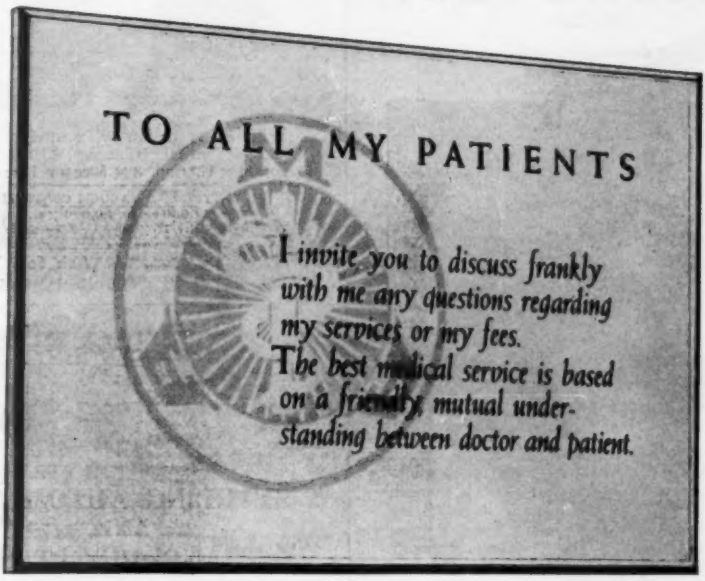
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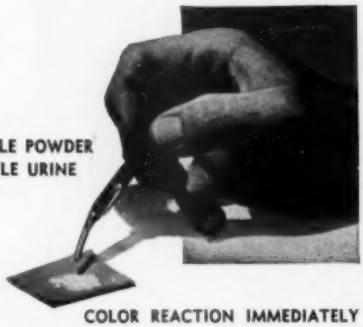


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With safety design self-centering device and dynamical balance. 13,000 r.p.m. 20,000 × G. Cap. 8 × 50cc or 15cc tubes.

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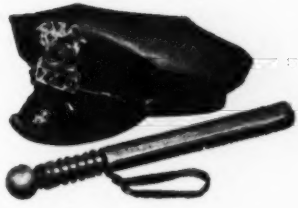

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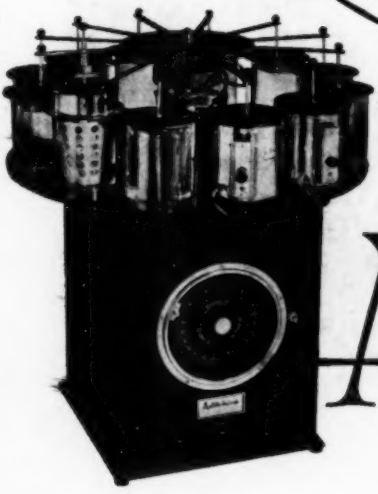

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